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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY WASHINGTON, D.C. 20460

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004679

OFFICE OF PESTICIDES AND TOXIC SUBSTANCE

MEMORANDUM:

S"BJECT: Toxicology Branch Chapter of the Registration

Standard for Benomyl

Tox. Chem. No.: 75A

TO:

H. Jacoby (PM 21)

Registration Division (TS-767C)

FROM:

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Attached is the Toxicology Branch (TB) chapter for the registration standard for benomvl including the following six subparts.

Generic data tables Policy discussion Data gaps Tolerance reassessment Bibliography Toxicology Branch "one-liners"

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POLICY DISCUSSION

A. Introduction

Benomyl, a systemic fungicide used in many agricultural and non-agricultural sites. The structure of benomyl (methyl-1(Butylcarbamoyl)-2-benzimidazolecarbamate) and its major metabolite MBC (methyl 2-benzimidazole carbamate) are:

The United States Environmental Protection Agency (EPA) issued a notice of Rebuttable Presumption Against Registration (RPAR) and Continued Registration of Pesticide Products containing Benomyl (Dec. 6, 1977) based on mutagenic effects and reduction in spermatogenic activity. The Position Document 2/3 (Aug. 39, 1979) determined that risks of concern included mutagenic effects, reduction in spermatogenic activity and acute toxicity to aquatic organisms. The Position Document 4 (Oct. 1, 1982) stated that new data indicated benomyl had oncogenic potential in mice.

As stated in the PD 1, 2/3 and 4, benomyl rapidly hydrolyses to MBC in an aqueous environment. MBC also appears to be the initial metabolite in mammalian systems. The acute and chronic toxicity of MBC is either similar or more severe than the parent compound, benomyl. For the above mentioned reasons, the Agency, in the PD 4 has used MBC data to confirm and supplement benomyl data where applicable.

The major regulatory action taken by the agency was to require the use of a dust mask by persons during mixing and loading of benomyl for aerial exposure. It was estimated that this would reduce the respiratory work-related component of exposure by 90 %. Dermal exposure was considered slight and not considered in the EPA risk estimate because of minimal dermal absorption (approximately 0.5 % per hour).

B. Use Summary

Benomyl is a systemic fungicide registered for many agricultural and non-agricultural uses. The crops and their respective tolerances will be discussed in the tolerance reassessment section. Non-agricultural uses include ornamentals and turf. Benomyl is available as a technical (95 %) and under the trade names of Benlate® and Tersan 1991® by E. I. duPont de Nemours and Co., Inc. Formulations include: formulation intermediate (FI; 50 %), granular (G; 1.1, 1.5, 1.57, 1.6 and 1.95 %), wettable powder (WP; 25 and 50 %), flowable concentrate (FIC; 0.25 lb/gal or 3 %, 75%(dry)), soluble concentrate/liquid (SC/L; 0.72 lb/gal or 10 %).

C. Toxicology Profile

1. Acute Effects

a. Acute oral toxicity (00097277)

10,000 mg/kg of technical benomyl was given by gavage to 10 each of male and female ChR-CD rats. There were no mortalities. The acute LD $_{50}$ was greater than 10,000 mg/kg.

Toxicity Category: IV

Core-Grade Classification: Minimum

The requirement for an acute oral study is satisfied.

b. Acute dermal toxicity (00064822)

Five each of male and female New Zealand white rabbits were treated dermally with 2000 mg/kg of Benlate* (75% a.i.). The substance was applied to shaved and abraded skin for 24 hr. There were no death within 14 days. The acute dermal LD_{50} was greater than 2000 mg/kg.

Toxicity Category: III

Core-Grade Classification: Guideline

The requirement for an acute oral study is satisfied.

c. l. Acute inhalation toxicity (00097599)

Six male rats were treated with either 0, .27, 1.39 or 4.01 mg/l (actual conc.) of benomyl, 50 % wettable powder. There were no deaths in the treatment groups. All groups however, had slight to severe aspermatogenesis. The acute inhalation LC50 was greater than 4.01 mg/l. There is no reason to expect the toxicity category to change with females.

Toxicity Category: III

Core-Grade Classification: minimum

Acute inhalation toxicity (00097281)

This study was designed to determine the NOEL for aspermatogenesis due to acute inhalation exposure. Ten male ChR rats were exposed for 4 hr to benomy1, 50 % wettable powder in an inhalation chamber at

^{*} Benomyl is the active ingredient

0, .02, .12, .20 or .82 mg/l. There were no deaths at any treatment level. Aspermatogenesis was present only in the 0.82 mg/l group (HDT).

Core-Grade Classification: minimum

These studies satisfy the requirement for data on acute inhalation toxicity.

d. 1. Primary eye irritation (00064820)

Benlate®Dry Flowable* (75 % a.i.), when applied to rabbit eyes, produced corneal opacities which were reversible by 11 days without irrigation. Mild iritis and conjunctivitis were present for only 3 days after treatment. Washing was effective in decreasing the severity and duration of the lesions.

Toxicity Category: II

Core-Grade Classification: Guideline

2. Primary eye irritation (00084579)

Benlate®Dry Flowable* (75 % a.i.), when applied to rabbit eyes, produced corneal opacities which were reversible by 7 days without irrigation. Mild iritis and conjunctivitis were present for only 3 days after treatment. Washing was effective in decreasing the severity and duration of the lesions.

Toxicity Category: III

Core-Grade Classification: Minimum

The toxicity category for eye irritation for labelling purposes is II since one study had corneal opacities present at 8 days, reversible by 11 days. These studies satisfy the requirement for data on primary eye irritation.

e. Primary skin irritation (00064821)

Six New Zealand white rabbits were exposed to Benlate®Dry Flowable* (75 % a.i.), in an aqueous paste for 24 hr. There was mild irritation which was reversible by day 6. The day 1 PIS was .67.

Toxicity Category: IV

Core-Grade Classification: Guideline

This study satisfies the requirement for data on primary skin irritation.

f. Dermal sensitization (00097289)

Benomyl technical was applied to the skin of 10 male guinea pigs. It was a mild to moderate sensitizer.

Core-Grade Classification: minimum

This study satisfies the requirement for data on dermal sensitization.

- Subchronic Toxicity (oral)
 - a. Subchronic oral rodent (00066771)

Benomyl, 70 % WP (wettable powder) (72.2 % a.i.) was incorporated into the diet of 4 ChR-CD rats per sex per group at the following concentrations; 0, 100, 500, and 2500 ppm for 90 days. The only toxic signs were increased relative and absolute liver weight (female) and increased SGPT (male) values, both in the high dose. The NOEL was 500 ppm. The LEL, based on SGPT and liver weight increases, was 2500 ppm.

Core-Grade Classification: minimum

b. Subchronic oral non-rodent (00066785)

Benomyl, 50 % WP (51.1 % tech.) was incorporated into the diets of 4 beagles per sex per group at the following concentrations, 0, 100, 500, and 2500 ppm for 90 days. Toxic signs included depressed albumen/globulin (A/G) ratio and increased SGPT in HDT males throughout the study. The NOEL was 500 ppm. The LEL, based on increased SGPT and a depressed A/G ratio, was 2500 ppm.

Core-Grade Classification: minimum

c. Subchronic oral non-rodent (00099130) (MBC)

MBC, 50 % WP (53 % tech.) was incorporated into the diets of 4 beagles per sex per group at the following concentrations, 0, 100, 500, and 2500 (lowered to 1500 at 3 weeks due to weight loss) ppm for 90 days. Toxic signs included increased alkaline phosphatase and SGPT in HDT males, increased cholesterol in mid and high dose males and females, decreased albumen in high dose males

^{*} Benomyl is the active ingredient.

and females. Absolute testicular weight was decreased in high dose males. One high dose male and female had hepatic cirrhosis and necrosis. One high dose male had diffuse testicular degeneration. The NOEL was 500 ppm. The LEL, based on altered liver function tests, liver histology, decreased testicular weight and testicular degeneration, was 2500 ppm.

Core-Grade Classification: minimum

These studies satisfy the requirement for data on subchronic oral toxicity in rodents and non-rodents.

- 3. Subchronic Toxicity (dermal) (00097287)
 - a. Benlate® (a.i. 51.5 %) was applied daily (5 out of every 7 days) for 3 weeks to the skin of New Zealand rabbits. The following doses were used: 0, 50, 250, 500, 1000 and 5000 mg/kg (based on % a.i.). Testicular weights (relative and absolute) were nonstatistically decreased at 1000 mg/kg and above. The NOEL was 500 and the LEL, based on decreased testicular weights, was 1000 mg/kg.

Core-Grade Classification: minimum

This study satisfies the requirement for subchronic dermal toxicity testing.

- 4. Subchronic Toxicity (inhalation)
 - a. Subchronic inhalation

There is currently no subacute inhalation study. This study is required for registration because:

1) farm applicators may receive repeated exposure to benomyl since large fields or orchards may require multiple days to complete fungicide application.

Custom applicators may be exposed daily for the length of the growing season. 2) The lowest effect level for spermatogenic inhibition in an acute rat inhalation study was 33 mg/kg (NOEL = 7.5 mg/kg). The margin of safety is only 21 since applicators (mixer/loader) may be exposed to as much as 0.35 mg/kg/day by the inhalation route (see PD4).

- 5. Neurotoxicity (GS0119-007)
 - a. Benomyl was tested in hens at 500, 2500 and 5000 mg/kg. There was no indication of delayed neurotoxicity.

This study satisfies the requirement for data on neurotoxicity.

Reproduction and Fertility Effects (feeding)

a. 3 Generation Reproduction Study - rats (00066773)

Benomyl 50 or 70 % WP (dose based on % a.i.) was administered in the diet at 0, 100, 500, and 2500 ppm to male and female ChR-CD rats for 3 generations (7 litters). Six males and females were mated for the first generation, 12 males and females for the second generation and 20 males and females for the third generation. Histology was performed on F_{3b} weanlings. F_{3c} pups were used for a post weaning growth curve study. No treatment related effects were seen with the exception of pup weanling weights in F_{2b} , F_{3b} and F_{3c} litters at 500 and 2500 ppm as compared to control values. The NOEL was 100 ppm and the LEL, based on decreased pup weanling weights, was 500 ppm.

Core-Grade Classification: minimum

This study satisfies the requirement for data on reproduction effects.

b. 3 Generation Reproduction Study - rats (00088333) (MBC)

MBC 50 or 70 % WP (dose based on % a.i.) was administered in the diet at 0, 100, 500, 2500 (raised to 10,000 ppm at 20 weeks), and 5000 ppm to male and female ChR-CD rats for 3 generations (6 litters). Sixteen males and females were mated for each generation. Histology was performed on F3b weanlings from 0, 500 and 10,000 ppm litters. No treatment related effects were seen with the exception of decreased weanling weights at 5000 and 10,000 ppm as compared to control values. The NOEL was 500 ppm (25 mg/kg) and the LEL, based on decreased pup weanling weights, was 500 ppm (250 mg/kg).

Core-Grade Classification: minimum

7. Teratogenicity

a. Rabbits (00035352)

Benomyl (50 % purity) was added to the diets of 15 New Zealand white albino rabbits at each of the following doses: 0, 100 and 500 ppm for days 8 through 16 of gestation. Fetuses were examined either after sacrifice on days 29 or 30 of gestation or after natural parturition. There were no treatment related fetal or maternal toxic effects

or teratogenic effects observed in any group. This study can not be used for regulatory purposes however, due to the dietary route of exposure and the absence of fetal or maternal toxicity at the high dose tested.

Core-Grade Classification: supplementary

This study does not satisfy the requirement for data on teratogenic effects in one species.

b. rat (GS0119-009)

Benomyl (99.2 % a.i.) was administered by gavage to 27 ChR-CD strain rats at each of the following doses: 0, 3, 10, 30, 62.5, and 125 mg/kg/day for days 7 through 16 of gestation. Dams were sacrificed on day 21 of gestation and the fetuses examined. There were no treatment related maternal or fetal toxic effects except for decreased fetal weight in the 62.5 and 125 mg/kg/day groups. There were significant increases in microphthalmia and anophthalmia at 62.5 and 125 mg/kg/day and distended lateral ventricles and hydrocephaly at 125 mg/kg/day. Two cases of microphthalmia also occurred at 10 mg/kg day. The registrant felt that this may be due to treatment since the background incidence of this lesion at the testing laboratory was 1/1000. The NOEL for maternal toxicity was greater than 125 mg/kg/day and the fetal toxic NOEL was 30 mg/kg/day. The teratogenic NOEL, however remains undetermined until the LEL for microphthalmia is determined. It is at least 62.5 mg/kg/day however.

Core-Grade Classification: supplementary

c. rat (00115674, 00126522)

Benomyl (99.1 % purity) was administered by gavage to 46-48 Crl:CD® (SD)BR rats at each of the following doses: 0.3, 6.25, 10, 20, 30 and 62.5 (only 19 dams) mg/kg/day for days 7 through 16 of gestation. Dams were sacrificed on day 21 of gestation and the fetuses were examined specifically for ocular effects. There were no treatment related signs of maternal toxicity noted. The high dose fetuses were significantly lighter than the controls. There was 1 fetus with microphthalmia present in the 16 litters in the high dose, no other ocular abnormali ties were reported.

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Studies 7.b. and 7.c., when considered together, are core-minimum. Together, they satisfy the requirement for data on teratogenic effects of benomyl on one species. The combined NOEL was 30 mg/kg/day and the LEL was 62.5 mg/kg/

d. mouse (GS0119-017)

Benomyl technical was administered by gavage to 25 pregnant CD-1 mice at each of the following doses: 0, 50, 100 and 200 mg/kg/day for days 7 through 17 of gestation. Dams were sacrificed on day 18 of gestation and the fetuses were examined. Doses as high as 200 mg/kg/day did not affect maternal viability or growth. Doses of 200 (significant from controls p < .05) and, to a much lesser extent, 100 mg/kg/day adversely affected fetal development including: decreased fetal weight, and delayed skeletal and visceral (including subnormal vertebral centrums, enlarged cerebral ventricles, and renal pelves) development, and increased supernumerary ribs. The incidence of major anomalies observed in the fetuses was 1.3, 1.0, 16.8 and 47.3 % at 0, 50, 100 and 200 mg/kg/day The incidences of major fetal and litter anomalies were significant at the p < .001 in the mid and high dose groups. Anomalies included: short and/or kinky tail, fused vertebrae, fused ribs and cleft palate. The NOEL was 50 mg/kg/day and the LEL, based on teratogenic effects, was 100 mg/kg/day.

Core-Grade Classification: minimum

This study satisfies the requirement for data on the teratogenic effects of benomyl in one species.

The requirement for data on teratogenic effects in two species has been satisfied.

8. Mutagenicity

a. Gene Mutation

- 1. (GS0119-001) Benomy1 (99.6 % a.i.), at levels of 500 and 10,000 ug/plate was mutagenic in S. typhimurium strains TA1535 and TA98 with activation. Classification: acceptable
- 2. (00038808) Chinese hamster ovary cells (HGPRT) were treated with from 17 to 172 uM of benomyl (99.9-100 % a.i.) with and without S-9 activation. Benomyl was not mutagenic in this system.

a

3. (GS0119-002) Benomyl was tested in mouse L5178Y TK+/- lymphoma cells with and without activation. The test material was mutagenic without activation at 50 ug/ml and with activation from 12 ug/ml to 25 ug/ml. At higher doses relative total cell growth was less than 10 %. At lower doses, mutagenic frequency was not consistently twice the control rate. Mutagenic activity was enhanced by activation.

Classification: acceptable

b. Chromosomal Aberrations

1. (GS0119-003) Micronucleus test - Benomyl (% a.i. not given) was administered by gavage to male mice on 2 consecutive days at the following doses: 0, 250, 500 and 1000 mg/kg/day. Animals were sacrificed 24, 48 or 72 hours after the second dose. Five hundred polychromatic erythrocytes (PCE) per rat from the femoral bone marrow were examined for micronuclei and the number of mature erythrocytes was counted until 200 PCEs were found. A compound is considered positive if the number of cells with micronuclei/500 PCEs is statistically increased over controls, in at least 2 dose-time groups. There were increased numbers of cells with micronucle in 4 groups including low and mid dose groups at 48 hr and the high dose group at 48 and 72 hr. Benomyl is considered positive in this test system.

Classification: acceptable

(GS0119-004) Sister chromatid exchange (SCE) -Chinese hamster ovary cells (in culture) were treated with from 0.625 to 10 ug/ml of benomyl (99 % a.i.) without activation or from 0.375 to 150 ug/ml of benomyl with activation. Cells underwent at least 2 cell divisions within 24 hr. Two samples of 25 cells were scored for SCE and chromosome number per treatment. There were not enough metaphases at concentrations of 5 ug/ml and above, without activation. Comparing the scores from 3 cytogeneticists using an analysis of variance indicated a small but statistically significant increase in SCEs with benomyl with and without activation. The authors concluded that benomyl was weakly positive in this test system.

Classification: acceptable

c. Other mutagenic testing, DNA repair

1. (GS0119-005) Mouse - Benomyl (% a.i. was unspecified) was tested for mutagenic effects in I° mouse (B6C3F1) hepatocytes using tritiated thymidine as the label. Concentrations ranged from 0.5 to .00005 mg/ml and were tested in triplicate Cells were fixed and examined microscopically for cytotoxicity then analyzed using autoradiography to determine tritium uptake. A net increase of 5 grains in each nucleus (above background) in all 3 replicates were considered a positive response. Level of benomyl 0.05 mg/kg and above, were cytotoxic. Benomyl did not induce DNA repair in this test system.

Classification: acceptable

2. (GS0119-006) rat - Benomyl (% a.i. was unspecified) was tested for mutagenic effects in I° rat (F344) hepatocytes using tritiated thymidine as the label. Concentrations ranged from 0.5 to 0.00005 mg/ml and were tested in triplicate. Cells were fixed and examined microscopically for cytotoxicity then analyzed using autoradiography to determine tritium uptake. A net increase of 5 grains in each nucleus (above background) in all 3 replicates were considered a positive response. Level of benomyl 0.05 mg/kg and above, were cytotoxic. Benomyl did not induce DNA repair in this test system.

Classification: acceptable

The requirements for mutagenic testing have been met.

Chronic Toxicity (feeding)

a. rat (00097284)

Thirty six albino Charles River CD rats of each sex were assigned to groups given 0, 100, 500, and 2500 ppm of 50 or 70 % benomyl WP (dose based on a.i.) for 2 years. There were no treatment related effects observed throughout the study. At sacrifice, there were no treatment related changes in neoplastic or non-neoplastic lesions, or changes in body weight or organ weights (including testicular weight). A maximum tolerated dose was not established. The NOEL for toxicity was established at 2500 ppm.

Core-Grade Classification: minimum for chronic toxici

b. Non-rodent (00097305, 00081913, 00097318, 00097326, 00061618)

Four beagles of each sex were assigned to groups given 0, 100, 500, and 2500 ppm of Benomyl (50 % a.i.) for 1 or 2 years. A fifth male added to the high dose group when I dog became sick. Body weight gain and food consumption were decreased in the high dose males and females. The following effects occurred in the 2500 ppm males: elevated cholesterol and alkaline phosphatase from 1 month to the end of the study; elevated glutamic-pyruvic transaminase activity levels which returned to normal by 15 months; decreased albumin/globulin ratio and total protein from 1 to 2 months to the end of the study. Hepatic cirrhosis, observed with both micro- and macroscopic examination, was observed in 1/2 males at 1 year (in 0/1 females), and 2/3 males and 1/3 females at 2 years. Testicular lesions, after examination by the E.P.A. pathologist, were considered unrelated to ingestion of the chemical. The NOEL was 500 ppm and the LEL was 2500 ppm based on biochemical alterations, hepatic cirrhosis, decreased weight gain and lower food consumption.

Core-Grade Classification: minimum

c. rat (00088333) (MBC)

Thirty six albino Charles River CD rats of each sex were assigned to groups given 0, 100, 500, and 2500 (raised to 10,000 ppm after week 20) 5000 ppm of 50 or 70 % MBC (dose based on a.i.) for 2 years. There were no treatment related effects observed throughout the study. At sacrifice, there were no treatment related changes in neoplastic lesions or organ weights. Mid dose females and high dose males and females weighed less than controls. Mid and high dose males and females had increased incidence of pericholangitis and cholangiohepatitis. Hematocrit (HCT), hemoglobin (HGB), and red blood cell counts were decreased in mid and high dose females; HCT and HGB were not significantly decreased in high dose males. The NOEL was 500 ppm and the LEL based on decreased weight gain, altered hematology and liver nistology was 5000 ppm.

Core-Grade Classification: minimum

d. Non-rodent (00088333) (MBC)

Four beagles of each sex were assigned to groups given 0, 100, 500, and 2500 lowered to 1500 ppm of MBC (53 % a.i.) for 2 years. Body weight gain and food consumption were decreased in the high dose males and females. The high dose males were sacrificed early due to poor nutrition. No females died early. High dose dogs developed anorexia, distended abdomens and poor condition. The following effects occurred in the 500 and 1500 ppm dogs: elevated cholesterol, BUN, total protien, SGPT and alkaline phosphatase. Hepatic cirrhosis, and mild chronic hepatitis was observed at 500 ppm and above. The NOEL was 100 ppm and the LEL was 500 ppm based on biochemical alterations, hepatic cirrhosis.

Core-Grade Classification: minimum

These studies satisfy the requirements for chronic toxicity testing in a rodent and non-rodent species.

10. Oncogenicity (feeding)

a. Rat (00097284) benomyl, see section 9.a.

Core-Grade Classification: supplementary for onco.

Rat (00088333) MBC, see section 9.c.

Core-Grade Classification: minimum for onco.

Although no maximum tolerated dose (MTD) has been established in a chronic/onco. rat study using benomyl (high dose of 2500 ppm), the MTD for the chronic/oncogenicity study with MBC was set at 5000 ppm. These studies satisfy part of the oncogenicity requirement for registration.

b. Mouse (00096514)

Eighty CD-1 mice of each sex were assigned to groups given 0, 500, 1500 and 7500 (reduced to 5000 ppm after 37 weeks) ppm of benomyl (99-99.2 % pure). There was decreased body weight throughout the study at the high dose and to a lesser extent the mid dose. There was increased relative liver weight in the high dose males and females as well were decreased in the high dose males. High dose males also had microscopic evidence of hepatocellular and testicular (and epididymal) degeneration. Under the conditions of this study, benomyl fed at a

minimum of 500 ppm demonstrated oncogenic potential in the liver and lung. Hepatocellular carcinomas were induced in both males (low and mid doses) and females (low, mid and high doses). The combined incidences of hepatocellular adenomas and carcinomas were statistically increased in the mid and high dose females, and low and mid dose males. Pulmonary alveologenic carcinomas were induced in males at the low and mid dose. Oncogenic potential at 500 ppm.

Core-Grade Classification: minimum

This study satisfies part of the oncogenicity requirements for registration.

The chronic rat and mouse oncogenicity studies satisfy the oncogenicity study requirements for registration.

c. Mouse (00096513) (MBC)

Eighty CD-1 mice of each sex were assigned to groups given 0, 500, 1500 and 7500 (males were reduced to 3750 ppm after 66 weeks due to mortality) ppm There were no treatment related changes in body weight, food consumption or clinical signs of toxicity throughout the study. High dose males were sacrificed at 73 weeks due to high mortality. High dose females had reduced erythrocyte and a marginal decrease in hemoglobin concentration. Abs. liver weight (high dose females) and rel. liver weight (mid and high dose females) were increased. Mid and high dose males had microscopic evidence of hepatotoxicity and necrosis. Hepatocellular carcinomas were increased in the mid dose males; too few male mice survived to 18 months at the high dose to ascertain the oncogenic potential at this dose level in males. Mid and high dose males and females had lymphoid depletion of the thymus. Under the conditions of this study, MBC was associated with an increased incidence of liver carcinomas in mid and high dose females. Hepatocellular adenomas were marginally increased in the low and mid dose females. The systemic NOEL was 500 ppm and the LEL, based on increased incidence of lymphoid depletion of the thymus in males and females, hepatotoxic lesions in males, was 1500 ppm. MBC demonstrated oncogenic potential at 1500 ppm (hepatocellular carcinomas).

Core-Grade Classification: minimum

c. Mouse (GS0119-011) (MBC)

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MBC was administered to 100-120 male and female NMRKf(SPF 71) mice per group at dosage levels of 0, 50, 150, 300 and 1000 (increased to 5000 after 8 weeks) ppm. Animals were sacrificed after 96 weeks. There was increased abs. and rel. liver weight, marked hepatocellular necrosis and cellular alterations indicative of hepatotoxicity at the high dose in both males and females. There was no increased incidence of hepatic neoplasia in treated as compared with control mice. The NOEL was 300 ppm and the LEL based on hepatotoxicity was 5000 ppm.

Core-Grade Classification: minimum

d. Mouse (GS0119-012) (MBC)

MBC was administered to 100 male and female SPF Swiss mice per group at dosage levels of 0, 150, 300, and 1000 (increased to 5000 ppm at 8 weeks) ppm for 80 weeks. Results were presented in summary form only. Body weight and condition were not affected by the compound. Rel. liver weights were "altered" in the high dose male and female groups. Clear cell and mixed cell foci were present in livers of the high dose males and females High dose females had an increase incidence of neoplastic nodules. High dose males however had an increased incidence of hepatoblastomas. The NOEL was 300 ppm and the LEL based on hepatic lesions and altered cell foci was 5000 ppm. MBC demonstrated oncogenic potential (hepatic neoplasia) at 5000 ppm.

Core-Grade Classification: supplementary

11. Metabolism - (00066776)

One male rat was treated with 2500 ppm benomyl (% a.i.unknown), for 12 days then given 7.7 mg/kg of ring labeled benomyl by gavage. The only urinary metabolites identified were sulphate and glucuronide conjugates of methyl 5-hydroxybenzimidazole-2-yl-carbamate (5-OH-MBC). Most of the label (greater than 99 %) was excreted by 72 hr, with the majority observed in the urine (86 %) and 13 % in the feces.

Core-Grade Classification: Unacceptable

Although some metabolic data in rodents is available (see policy discussion), there are currently no low dose, high dose or repeated dose metabolism studies meeting our core-minimum requirements of acceptability. These studies are needed to satisfy the requirements for registration.

12. Dermal absorption (GS0119-014)

Four rats/time point/dose were treated dermally (greater than 16 % of their surface area) with *Benlate*WP (50 % a.i.). The duration of exposures were 0.5, 1, 2, 4, and 10 hours, doses tested were 0.2, 2, 20, and 200 mg of 14C-Benlate/rat (0.1, 1, 10, and 100 mg, respectively of benomyl). The concentration of benomyl in the blood increased (nonlinear) with increasing dose (see table 1). The percent of administered dose of benomyl in the urine decreased (nonlinear) with increasing dose and increased (nonlinear with duration. The percent absorption also had a nonlinear decrease with increasing dose and a nonlinear increase with duration (see table 2). The percent absorbed ranged from 0.031 (high dose) to 3.518 (low dose) for the maximum exposure of 10 hours. By 10 hours 96-99 percent of the absorbed dose at all treatment levels had been excreted in the urine.

Table 1. Cor	centra	ation of ben	omyl in	the blood	(ug/ml)
Dose (a.i.) mg/rat	0.5	Duration of 1.0	exposui 2.0	e (hours)	10
0.1	.001	.004	.004	.004	.003
1.0	.006	.009	.008	.008	.004
10	.026	.028	.034	.036	.024
100	.033	.054	.048	.070	.064

Table 2. Total percent of dose of benomyl absorbed										
Dose (a.i.)		Duration of		*	•					
mg/rat	0.5	1.0	<u> </u>	4.0	10					
0.1	.046	.174	.714	1.734	3.518					
1.0	.016	.045	.118	.340	.491					
10	.006	.011	.039	.046	.096					
100	.001	.004	.010	.029	.031					

Classification - acceptable

D. Policy Discussions

The toxicity concerns have been evaluated in detail in the Position Document 2/3 (PD 2/3) (Dec. 1977) and 4 (Oct. 1982). The risks are based on the increase in tumors, as well as increased teratogenic, mutagenic and spermatogenic effects related to benomyl or its metabolite, MBC.

Although valid metabolism studies have not been performed to adequately describe the metabolism of benomyl in animals, one postulated pathway reported in an article by Douche (00036818) is as follows:

Fig. 3. Metabolic pathway of Benomyl in mice, rabbits and theep.

- a. 2-aminobenzimidazole (2AB)
- b 5 hydroxy-2-aminobenzimidazole (5 OH-2-AB)
- c. methyl benzimidazole-2ylcarbamate (MBC)
- e. methyl 5-hydroxybenzimidazole-2-yl-carbamate (5 OH-MBC)
- f. Benomyl (parent)

Animals all received 0.1 gm/kg (substantially higher than the estimated dietary exposures) of compound either by oral (P.O.) or intraperitoneal (I.P.) route of exposure. He reported that metabolite distribution in the mouse, rabbit and sheep was similar. It was also similar whether administered orally or peritoneally suggesting biliary secretion as part of the process. Ninety-four % of the label was excreted by mice within 96 hr with 20 % of the administered label excreted as conjugates (sulfate and glucuronide) of hydroxylated metabolites (44-71 % in urine; 21-46 % in feces). There was no parent compound found in either the urine or feces. The metabolic pathway with 0.1 gm exposure may not however, represent the pathway that would occur with lower dietary exposure levels.

In a separate study (00066776), a single male rat received 2500 ppm of cold benomyl for 12 days followed by a dose of 14C labeled benomyl (7.7 mg/kg). The only metabolite found in the urine, after treatment with glucuronidase and sulfatase, was 5 OH-MBC. Again most of the label was observed in the urine (86 %), 13 % in the feces. Less than 1 % remained in the carcass.

The metabolism of benomyl needs to be more completely described. Studies need to be conducted in males and females using sufficient numbers of animals at: 1) expected exposure levels, 2) elevated levels and 3) with pretreatment, in order to have adequate information to satisfy the regulations.

Table 1. Incidence of Liver Tumors in Mice from Positive Studies

Chunin		1		se in ppr			3750/		
Strain	neopl.	0	150	300	500	1500	7500**	5000	7500
Benomyl 00096514									
CD-1-male	benign malig. total	9/77 16/77 25/77			9/80 26/80 35/80	11/79 41/79 52/79		10/80 17/80 27/80	
-female	benign malig. total	2/77 2/77 4/77			2/80 7/80 9/80	7/79 6/79 13/79		7/77 14/77 21/77	
MBC									
00096513	benign				15/80	14/80	3/80*		
CD-1-male	malig.	2/80			5/80	9/80	0/80*		}
	total	13/80			20/80	23/80	3/80*		
-female	benign malig.	0/79 1/79			5/78 4/78	5/80 15/80			3/78 12/78
1	Hb	0/79		i	0/78	1/80			0/79
	total	1/79			9/78	21/80			15/78
MBC		",			2,,,,	112,000			20, 10
GS0119-012	NN	9/100	6/98	13/100				9/100	
Swiss-male	malig.	1/100	1/98	2/100				1/100	
	Hb	1/100	1/98	1/100				7/100	
	total	10/100	8/98	16/100				17/100	
-female	NINI	0/97	1/99	1/98				9/97	
	malig.	1/97	0/99	0/98				0/97	
	Hb	0/97	0/99	0/98				0/97	
	total	1/97	1/99	1/98					
	wear	1/3/	1/99	1/ 90				9/97	

liver neopl.

benign (adenomas)

malig. (adenocarcinomas)

N.N. (neoplastic nodules - benign) Hb (hepatoblastoma - malignant)

^{*}sacrificed at week 73 due to high mortality
**Males received 7500 ppm for 15 months, than 3750 ppm until sacrifice

The positive <u>oncogenicity</u> studies discussed in the PD4 included one benomyl and two MBC mouse studies. Both the benomyl (00096514) and 1 MBC study in CD®1 mice (00096513) had an increased incidence of hepatocellular adenomas and carcinomas in males and females (see table 1).

Beems et al. (GS0119-012) reported in 1976 that SPF, albino, Swiss random bred mice treated with up to 5000 ppm for 80 weeks, had an increased incidence of malignant liver tumors (high dose males) and benign liver tumors (high dose females) (see table 1).

A recently submitted study tested MBC in NMRKf(SPF71) mice for 22 months (GS0119-011). A preliminary evaluation indicated no increase in incidence of neoplasia. However, there was an increased incidence in toxic liver damage consisting of hypertrophy of centrolobular and intermediary hepatocytes, liver necrosis and increased mitotic activity at the high dose (5000 ppm).

There was no increased incidence of neoplasms in dogs or albino Charles River CD rats. Although, there was no maximum tolerated dose (MTD) established in the chronic benomyl rat study (high dose 2500 ppm), the MBC chronic mouse study established an MTD at 5000 ppm. It is expected that the toxicity of MBC is either equal to, or greater than benomyl since benomyl rapidly hydrolyzes to MBC both in vitro and in vivo in an acueous environment.

in vivo in an aqueous environment.

The Q₁* of 2.065 x 10⁻⁵, used in the PD4, was based on the hepatic neoplasms in CD-1 female mice treated with MBC since that study was considered to produce a more conservative assessme of risk than the benomyl study. The Toxicology Branch Peer Review Committee on Benomyl is currently evaluating the weight-of-the-evidence for assessing the oncogenic potential of benomyl.

The <u>mutagenic activity</u> of benomyl and MBC was discussed in detail in the PD 2/3 and 4. The PD 4 concluded that both compounds were spindle poisons which could result in nondisjunction and aneuploidy. Nondisjunction was reported in A. nidulans after exposure to benomyl and MBC (GS0119-013). Benomyl was associated with gene mutation in strains of S. typhimurium with activation (GS0119-001) at 10,000 ug/plate. It also was mutagenic in mouse lymphoma cells (L5178Y TK+/-) (GS0119-002) with and to a lesser extent without activation. In chinese hamster ovary cells (HGPRT) (00038808) however, benomyl, even at levels of 172 uM, was not mutagenic with or without activation. Benomyl was not associated with DNA repair in primary mouse and rat hepatocyte cultures (GS0119-005, GS0119-006). There were no chromosome breaks in vivo with chinese hamster bone marrow cells treated with up to 1000 mg/ml MBC (GS0119-008). Although benomyl and MBC caused increased incidence of micronuclei in polychromatic erythrocytes in mouse bone marrow it is likely that this response was a result of spindle effects rather than chromosomal damage (GS0119--008). Benomyl was weakly positive for sister chromatid exchange (SCE) in vitro in chinese hamster ovary cells with and without 19 activation (GS0119-004). The PD4 concluded that "benomyl and the MBC metabolite of benomyl ... have been shown to cause effects to the cellular spindle apparatus. The impact of this effect to human health cannot be adequately assessed at this time. Therefore, mutagenic risk in the form of heritable spindle effects or point mutagenicity do not now lead to a recommendation for regulatory action. However, the data on mutagenicity are supportive of the qualitative determination of benomyl's potential teratogenic, spermatogenic and oncogenic effects."

The testes has been identified as a primary target of benomyl. It has been reported to affect the testes and epididymus in both acute and chronic studies. Decreased size of testes, depressed spermatogenesis or aspermatogenesis has been observed in rats, dogs, mice and rabbits by oral (gavage and dietary), dermal and inhalation routes. These studies are summarized in Table 2.

Table 2 Benomyl Studies With Testicular Effects

Oral - Dietary

Dogs - no effect after 2 years at 2500 ppm (62.5 mg/kg/day) (00097305, 00081913, 00097318, 00097326, 00061618, 00068981).

Rats (ChR-CD) - no effect in rats after 2 years at 2500 ppm (125 mg/kg/day)(00097284, 00068981).

Oral - intubation

Rats (ChR-CD) - single exposure - testicular effects after
14 days with 670 mg/kg (NOEL = 450 mg/kg) (00066779).
Rats (ChR-CD) - 10 doses (in 14 days) - testicular effects
at 200 mg/kg/day, the lowest dose tested (00097601).

Inhalation

Dermal

Rabbits - a 21 day exposure study resulted in decreased testicular weights at 1000 mg/kg, the NOEL was 500 mg/kg (00097287).

The teratogenic effects of benomyl were discussed in detail in the PD4. Microphthalmia, anophthalmia and hydrocephalia occurred when benomyl was administered by gavage to Wistar rats at 125 mg/kg/day on days 1-20 or 7-15 of gestation (NOEL = 62.5 mg/kg/day) (GS0119-015). In a separate gavage study with Wistar rats, lack of eye bulges and CNS herniations were observed at the low dose tested of 62.5 mg/kg/day (GS0119-016). Microphthalmia was also observed in the fetuses of ChR-CD rats given 62.5 mg/kg/day but not 30 mg/kg/day by gavage (00115674).

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This study was used to set the NOEL of 30mg/kg/day for teratogenesis. Benomyl was also teratogenic (LEL 100 mg/kg/day) in CD-I mice (GS0119-017) when administered by gavage. Studies in the rabbit and Wistar and ChR-CD rats have all been negative when the route of exposure to benomyl was dietary (00035352, GS0119-017, 00078620). The PD4 considered the different teratogenic results depending on exposure route as follows:

"The FIFRA Scientific Advisory Panel (SAP) has acknowledged the contradictory results obtained from the two methods of dosing and have recommended that any NOEL established through the gavage method of administration of benomyl should be qualified by the essentially negative

results obtained in dietary studies.

The Agency considers that gavage administration of test material is scientifically more acceptable than dietary dosing for determining a NOEL for teratogenicity because it eliminates problems of palatability, drug stability, nutrient integrity and calculations of accurate dose The Agency supports the ... opinion, that gavage assures relevance of treatment to the human condition for chemicals like benomyl (which are rapidly metabolized), since rodents, by preference, eat frequently during waking hours, whereas humans dine at relatively orderly intervals during the day. Therefore, the peaking of blood residues following gavage administration more nearly parallels the human situation than continuous uptake of the chemical in the rat diet."

The dermal absorption of benomyl is complex. Percent of benomyl absorbed/unit time decreased with increasing dose in a nonlinear fashion. It also increased with duration of exposure to a maximum at about 4 hours (see table). Blood concentrations of benomyl also increased with increasing dose (see table 3). Between 96 and 99 % of absorbed benomyl were excreted in the urine by 10 hours.

Percent of the benomyl dose absorbed per hour, Table 3.

and blood levels at 4 hours Dose (a.i.) Duration of exposure (hours) ug/ml in mg/rat 0.5 2.0 10 1.0 4.0 blood .090 .174 .357 0.1. 434 .352 .004 .085 1.0 .032 .045 .059 .049 .008 10 .012 .011 .020 .012 .010 .036 100 .002 .004 .005 .007 .003 .070

Benomyl has been shown to be a Toxicity Category II for eye irritation (00064820), a skin sensitizer (00097289) and a mild dermal irritant (toxicity category IV). The labels should have the appropriate warning statements.

Data Gaps for Benomyl

Subchronic inhalation toxicity study - 90-day

Metabolism studies: low dose, high dose, and repeated dose.

Tolerance Reassessment

Tolerances for benomyl in the CFR \$ 180.294 are for the parent compound and its metabolites containing the benzimidazole moiety (calculated as benomyl).

ADI

The five studies required to determine an acceptable daily intake (ADI) are listed in table 4.

Table 4. 5 required studies and NOELs for establishing an ADI

IGDIO 4. D redarred pres.	LOS WILL	10000 202 000	dollaring dir nor
Study Type	NO		Reference
	ppm	mg/kg/day	
chronic rat feeding	2500	125	00097284, 00068981
chronic dog feeding	500	12.5	00097305, 00081913, 00097318, 00097326, 00061619
3 generation reproduction	100	5 30	00066773 00115674, 00126522
rat teratology mouse teratology		50 50	GS0119-017

The no observable effect level (NOEL) of $\frac{5 \text{ mg/kg/day}}{1}$, obtained from the most sensitive study (see table 1), was divided by a safety factor of 100 to derive a ADI of $\frac{0.05 \text{ mg/kg/day}}{1}$. The maximal permissible intake (MPI) for a 60 kg adult is: 0.05 mg/kg/day x 60 kg = 3 mg/day

TMRC

Tolerances for the commodities in table 5 have been published in the federal register and have a theoretical maximal residual concentration (TMRC) of 1.9785 mg/day of benomyl which is 65.78 % of the ADI. The TMRC of 1.9785 mg/day would be 0.03289 mg/kg/day for a 60 kg adult. Table 6 lists commodities that have petitions pending for tolerances. These commodities, if approved, would add 0.2728 mg/day, raising the TMRC 13.79 % to 2.2513 mg/kg, which is 75.04 % of the ADI. The new TMRC of 2.2513 mg/day would be 0.03752 mg/kg/day for a 60 kg adult. The proceeding calculations used tolerances, the worst case for dietary exposure and did not consider per cent of crop treated and actual residue values (including processing).

treated and actual residue values (including processing).

The PD 4 listed percent of crop treated where the data was available (see table 5). When these values are taken into consideration the average residue level of benomyl (for crops with published tolerances) in the diet decreases to 1.00689 mg/day which is 33.56 % of the ADI.

Teratogenesis

The NOEL for teratogenesis used for this analysis was 30 mg/kg/day (00115674, 00126522). Tolerance level residues were assumed for every commodity with published tolerances. Using the detailed acute analysis 1 , with all statistics based on users daily consumption of food containing benomyl (for females 13 years of age and older) the weighted-average daily exposure was 0.039421 mg/kg/day. The MOS 2 based on the NOEL of 30 mg/kg/day was $\frac{30 \text{mg/kg/day}}{0.039421 \text{mg/kg/day}} = 761$.

The distribution of exposure for the population at risk (females greater than 13 years of age) using the TAS analysis, is summarized as follows. Forty four % of these women had a MOS at or above 1000. The NOEL was 200 times the exposure level reported for the individual with the highest potential estimated exposure.

Individual crops with high residues were tomatoes, brussel sprouts, grapefruit, oranges, peaches, nectarines, plums, pineapple.

Spermatogenic effects

Tolerance level residues were assumed for every commodity with published tolerances. The NOEL for spermatogenic effects used in the PD4 was 7.5 mg/kg/day was based on an acute rat inhalation study (00097281) because it was the most sensitive test for spermatogenic effects. The limiting dose used for comparison was 0.075 mg/kg/day (the NOEL of 7.5 mg/kg/day divided by a safety factor of 100). Using the TAS detailed acute analysis1, with all statistics based on users daily consumption of food containing benomy1 (for males 13 years of age and older) the weighted-average daily exposure was 0.035151 mg/kg/day. This was 46.87 % of the limiting dose of 0.075 mg/kg/day. The MOS² based on the NOEL of 7.5 mg/kg/day was 7.5mg/kg/day = 213

O.035151 mg/kg/day

The distribution of exposure for the population at risk (males greater than 13 years of age) using the TAS acute exposure analysis is summarized as follows. Thirteen % of these men had exposure at or above the limiting level. No individuals had exposures greater than 3 times the limiting dose. The MOS was 33 for the individual with the highest exposure.

Individual crops with residues which result in acute single serving dietary exposure to benomyl at or above 75 % of the limiting dose (0.075 mg/kg/day) were grapefruit, peaches, and pineapple.

The chronic dog feeding study (00097305) with a NOEL of 62.5 mg/kg/day (HDT) for spermatogenic effects, is the most conservative NOEL for this lesion, from a chronic feeding study (rat NOEL = 125 mg/kg/day; mouse NOEL = 225 mg/kg/day). The TMRC (calculated from the maximum published tolerances and

¹ Tolerance assessment system (TAS) was used for these calculations
2 MOS = margin of safety

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food factors) for chronic exposure to benomyl is 1.9735 mg/day (60 kg adult) or .03289 mg/kg/day. The resultant MOS using this NOEL (62.5 mg/kg/day) is $\frac{62.5 \text{ mg/kg/day}}{0.03289 \text{ mg/kg/day}} = 1900.$

Oncogenesis

The potency estimator, Q_1^* for oncogenicity, presented in the PD 4 was 2.065×10^{-3} (mg/kg body weight/day⁻¹) (see policy discussion). The upper 95 % bound on cancer risk for dietary exposure is 6.8×10^{-5} (the Q_1^* multiplied times the TMRC). Table 5 gives the risks for the individual commodities. When the percent of crops treated is taken into consideration the risk decreases to 3.5×10^{-5} . Both values however, round to 10^{-5} to 10^{-4} . Therefore, current data concerning percent of crops treated, does not appreciably decrease the dietary risks of benomyl.

The 3C2B letter of May 14, 1985, required the registrant to submit real residue on apples, peaches, pineapples, rice, milk, soybeans, citrus and tomatoes. The potential residues for these commodities, when based on published tolerances, comprise $\frac{76 \text{ }\$}{\$}$ of the TMRC. The resultant residue data may decrease the $\frac{1}{\$}$ ADI, teratogenic, spermatogenic and oncogenic risks from benomyl.

The risk to applicators and mixer/loaders was addressed in detail in the PD4. The Exposure Assessment Branch chapter has not yet been completed as of the date of this section. They have reported no major alterations in the use patterns of benomyl since the PD4 (verbal communication with Harry Day, EAB, 10/4/85). The worst case job related exposure would be for mixer/loaders for grapes/fruit crops with aerial application; 0.35 mg/kg/day without a dust mask. This results in a margin of safety of 21 for spermatogenic inhibition (NOEL from an acute rat inhalation study was 7.5 mg/kg). The exposure would decrease by 90 % with a dust mask. If alterations are reported in the Exposure Assessment chapter when completed, Toxicology Branch would need to consider their impact on the job related risk due to benomyl.

TABLE 5. Dietary "Worst Case" Exposure and Estimate of Upper Bound Risk (95 % Confidence Level) Based on Tolerances for Benomyl

	molowaneo.	Food factor	Daily Intake	Cancer Dick	Percent	Daily
CROP		room ractor	(mg/1.5kg	(100 % crop		adjust
Crop	(ppm)		diet/day)	treated)		% treat
			diec/day/	rreared)	creaced	& CLEA
cus fruits	10.	3.81	0.57179	10-5	66.	0.377 :
les	7.	2.53	0.26565	10-5	23.	0.061
atoes	5.	2.87	0.20503	10-6-10-5	18.	0.037
ches	15.	0.90	0.20235	10-6-10-5	74.	0.149
espple	35.	0.30	0.15560	10-6-10-5	33.	0.0513
		0.45	0.06745	10-6	29.	0.019
pes, not raisins	10.	2.04	0.06120	10-6	37.	0.0226
15	2.	28.62	0.06120	10-6	100.	0.0426
< & dairy prod.	0.1	0.55	0.04232	10-6	22.	0.0091
3	5.			10-6	100.	0.031
at	0.2	10.36	0.03109	10-6	29.	0.008
sins	50.	0.04	0.03066	10-6	80.	0.024
ons	1.1.	2.	0.03005	10-6	17.	0.0050
ns, inc prunes	15.	0.13	0.02989	10-6	46.	0.012
rs	7.	0.26	0.02683	10-6	73.	0.012
icots	15.	0.11	0.02529	10-7-10-6	36.	0.023
cries	15.	0.10	0.02299	10-7-10-6		0.000
t, inc poultry	0.1	13.85	0.02077	10 7-10 6	100.	
awberries	5.	0.18	0.01380	10-7-10-6	70.	0.009
∍ry	3.	0.29	0.01288		88.	0.011
ar, cane & beet	0.2	3.64	0.01091	10-7-10-6	100.	0.010
umbers, inc pickl	1.	0.73	0.01088	10-7-10-6	44.	0.004
seel sprouts	15.	0.03	0.00675	10-7	100.	0.006
tarines	15.	0.03	0.00675	10-7	74.	-0.005
nese cabbage	10.	0.03	0.00450	10-7	100.	0.004
delion	10.	0.03	0.00450	10-7	100.	0.004
nrooms	10.	0.03	0.00450	10-7	50.	0.002.
n, sweet	0.2	1.43	0.00429	10-7	100.	0.004.
anas	.2	1.42	0.00426	10-7	100.	0.004.
3	0.1	2.77	0.00416	10-7	100.	0.004
ckberries	7.	0.03	0.00315	10-7	50.	0.001
eberries :	7.	0.03	0.00315	10-7	55.	0.001
senberries	7.	0.03	0.00315	10-7	50.	0.001
rants	7.	0.03	0.00315	10-7	100.	0.00 3
berries	7.	0.03	0.00315	10-7	50.	0.001
anberries	7.	0.03	0.00315	10-7	50.	0.001
pberries	7.	0.03	0.00315	10-7	27.	0.000
beans (oil)	0.2	0.92	0.00275	10-7	2.6	0.000
nip greens	6.	0.03	0.00270	10-7	100.	0.002
bage, sauerkraut	0.2	0.74	0.00221	10-8-10-7	100.	0.002
pkin, inc squash	1.	0.11	0.00169	10-8-10-7	90.	0.001
rots	0.2	0.48	0.00144	10-8-10-7	100.	0.001
cados	3.	0.03	0.00135	10-8-10-7	5.	0.000
goes	3.	0.03	0.00135	10-8-10-7	50.	0.000
•	3.	0.03	0.00135	10-8-10-7	100.	0.001
ayas			0,00200			

mg/l.5kg diet/day

Table 5 continued

CROP	Tolerance (ppm)	Food factor	Daily Intake (mg/l.5kg diet/day)	Cancer Risk	of crop	Daily Inta adjusted f % treated
t potatoes	0.2	0.40	0.00120	10-8-10-7	100.	0.00120
_	0.2	0.36	0.00107	10-8-10-7	100.	0.00107
ıts	0.2	0.36	0.00107	10-8-10-7	22.	0.00024
∍r squash	1.	0.03	0.00045	10-8	100.	0.00045
∍rsquash	1.	0.03	0.00045	10-8	100.	0.00045
ers	0.2	0.12	0.00037	10-8	100.	0.00037
coli	0.2	0.1	0.00031	10-8	100.	0.00031
	0.2	0.10	0.00031	10-8	38.	0.00012
ards	0.2	0.08	0.00025	10-8	100.	0.00025
iflower	0.2	0.07	0.00021	10-9-10-8	100.	0.00021
ard greens	0.2	0.06	0.00018	10-9-10-8	100.	0.00018
ach	0.2	0.05	0.00015	10-9-10-8	100.	0.00015
ips	0.2	0.05	0.00015	10-9-10-8	100.	0.00015
ΣÝ	0.2	0.03	0.00009	10-9-10-8	100.	0.00009
lant	0.2	0.03	0.00009	10-9-10-8	100.	0.00009
ic	0.2	0.03	0.00009	10-9-10-8	100.	0.00009
	0.2	0.03	0.00009	10-9-10-8	100.	0.00009
:abi	0.2	0.03	0.00009	10-9-10-8	100.	0.00009
:	0.2	0.02	0.00009	10-9-10-8	100.	0.00009
agas	0.2	0.03	0.00009	10-9-10-8	100.	0.00009
	0.2	0.03	0.00009	10 <u>-9</u> -10 <u>-8</u>	100.	0.00009

1/1.5kg diet/day

TABLE 6. Dietary "Worst Case" Exposure and Estimate of Upper Bound Risk (95 % Confidence Level) Based on Proposed Tolerances for Benomyl

CROP	Tolerance (ppm)	Food factor	Daily Intake (mg/1.5kg diet/day)	Cancer Risk
yams (yautia) lettuce eggplant* peppers* escarole/endive	0.2 10. 4.8 4.8	0.03 1.31 0.03 0.12 0.03	0.00009 0.19622 0.00216 0.00883 0.00450	10-9-10-8 10-7-10-6 10-8-10-7 10-7-10-6 10-7
beets beet greens	0.2 15.	0.17 0.03	0.00052 0.00675	10 ⁻⁸ 10 ⁻⁷
liver* cabbage, sauerkraut*	1.8	0.3 0.74	0.00081 0.05298	10 ⁻⁸ 10 ⁻⁶

^{*}these represent additional tolerances over that previously published, i.e. eggplant: previous 0.2 ppm; additional requested 4.8 ppm; total new tolerance requested 5.0 ppm.

Tox Chem No. Benomyl 75A		EPA	File Last Updated	Current Date	
Study/Lab/Study #/Date	Material	Accession No.	Results: ID50, IC50, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
21—day dermal — rabbit; Haskell lab.;211—69; 7/20/69	<u>5</u> 2.5-53 % a.i.	MRID 00057287	NOEL = 500 mg/kg LEL = 1000 mg/kg based on a non- statistically sig. decr. in rel.& abs. testes weight lévels tested 50 to 5000 mg/kg		minimum 004679 —
Acute oral LD ₅₀ - rat Haskell labs; 17-69; 1/22/69	Technical	MRID 00097277	LD ₅₀ > 10,000 mg/kg	2	000721 Minimum 004679 -
Acute inhalation LC ₅₀ - rat; Hazleton Lab; #201- 220; 10/18/68	50% a.i. Fungicide 1991 Bencmyl WP	MRID 00097599	LC ₅₀ > 4.01 mg/L (HDT)(testicular alterations noted at all levels tested: 0.27, 1.0 and 4.01 mg/L)	Ħ	000721 0004678 minimum 004679 -
Primary dermal irrit guinea pig; 84-69; 4/18/69	technical	Acc.# 050427-W MRID 00097289-	mild skin irritation	NI .	minimum 004679 –
Dermal sensitizationguinea pig; 84-69; 4/18/69	technical	Acc.# 050427-W MRID 00097289	mild to moderate sensitization		minimum 004679 –
90-Day feeding - rat; Haskell Lab.; #11-67; 1/31/67	70% WP (72.2% tech)	MRID 00066771	Systemic NOEL = 500 ppm LEL = 2500 ppm based on incr. SGPT (male),rel. & abs. liver wt.(female) dose levels:0, 100, 500, 2500ppm(ai)		000721 004678 minimum 004679 -
90-Day feeding - dog; Haskell Lab.; #269-68; 11/20/68	51% Technical 50 % WP	MRID 00066785	Systemic NOEL = 500 ppm LEL = 2500 ppm based on incr. SGPT, Alk.phos, A/G ratio (male) dose levels: 0, 100, 500, 2500ppm(ai		000721 K5 004678 C3 minimum A3 004679 - C
2-Year feeding - rat; Haskell Lab.; #232-69; 8/15/69 (supp. path. report 66-77; 2/9/78)	51 or 72.2 % Tech, 50 or 70% WP	MRID 00097284 00068981	Systemic NOEL > 2500 ppm Oncoyenic NOEL > 2500 ppm No effect on sporm production Dosaye levels = 100, 500, 2500 ppm in ChR-CD strain		000721 004678 minimum chronc \$8486790 <u>n</u> ∞

**		. A		1 11/1/12			1955 J.	
	OORE Grade/ Doc. No.	000721 004678 minimm 004679 -	000721 004678 minimum 004679 –	000722 supplementary 004679 -	000722	Guideli (1000863 (1004679 -	Guideline 000863 004678 004679 -	Onideline 000863 0008679 004679
Current Date	TOX Category	-				111	Ħ	ZI.
File Last Updated	Results: LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL	Systemic NOEL = 500 ppm Systemic LEL = 2,500 ppm (HDT, cirrhosis and body weight depression Dosage levels = 100, 500, 2500 ppm	Systemic NOEL = 100 ppm LEL = 500 ppm based on decreased pup weights dose levels:0, 100, 500, 2500 ppm(ai)	Terata NOEL = 500 ppm (HDF) NOEL fetal, maternal tox > 500 ppm Dose levels: 0, 100, 500 ppm by diet	Fetotoxic NOEL = 62.5 mg/kg Fetotoxic LEL = 125 mg/kg Terata NOEL = 62.5 mg/kg Terata LEL = 125 mg/kg (Brain hernias, hydrocephaly and microphtalmia) Dosage = 62.5, 125, 250, 500 mg/kg (gavage) in Wistar strain	LD ₅₀ > 2000 mg/kg Severe skin irritation.	Corneal opacity at 8 days. For the irrigated eyes, irritation cleared by day 8. PIS day 1 = 28, day 11 = 0	Slight edema and slight erythema at 24 hours, at 72 hours, only very slight erythema. PIS = 0.67 All scores were Ø by day 6.
EPA	Accession No.	MRID 00097305 00081913 00097318 00097326 00061618 00068981	MRID 00066773	MRID 00035352	\$20119-015	243043 MRID 00064822	Acc.# 243043 MRID 00064820	Acc.# 243043 MRID 00064821
	Material	51 or 72.2 % Tech, 50 or 70% WP	51 or 72.2 % Tech, 50 or 70% WP	53.5% WP 50% a.i.	Technical	Benomyl – 75% (Benlate DF)	Benomyl – 75% (Benlate DF)	Bencmyl - 75% (Benlate DF)
Tox Chem No. Benomyl 75A	Study/Lab/Study #/Date	2-Year feeding - dog; Haskell Lab.; #48-70 (129-69,74-77), 48-70,66-77; 3/7/70	3-Generation reproduction - rat; Haskell Lab.; #264-68; 11/18/68	Teratology – rabbit; Hazleton Lab.;Hazelton; 210-214; 1968	Teratology – rat; Schtenberg & Torchinsky; 1972	Acute dermal LD ₅₀ - rabbit; Haskell; #554-80, 7/23/80	Primary eye irritation - rabbit; Haskell; #497- 80; 6/13/80	Primary dermal irrita- tion - rabbit; Haskell; #367-80;5/12/80

	CORE Grade/	Supplementary	002578 Upgraded to Minimum 003042	003726 minimum 004679 -	003728	003728	003728	034679
Current Date	XOT .	Category						
File Last UpdatedC	Results:	STUDY LIMITED TO MICHOPHTHALMIA NOEL = 30 mg/kg	Levels tested by gavage - (0, 3, 6.25, 10, 20, 30 & 62.5) ChR-CD rats	Oncogenic NOEL < 500 ppm male and female, significant increase in hepatocellular neoplasms in male and female, pulmon, alveol, carcin, in males; degen, of testes and epididymis at 5000 ppm. Dosage levels = 500, 1500, 5000 ppm (5000 lowered from 7500 ppm) in CD-1	GS0119-018 Terata NOEL = 5000 ppm (HDT) (in-MRID conclusive result since ingested 00078620 dose not measured accurately)ChR-CD	doses 0, 100, 500, 2500, 5000 ppm GS0119-016 Terata NOEL < 62.5 mg/kg (LDT; CNS herniations, defects of extremities, lack of eye bulges) Dosage levels = 0 - 500 mg/kg/day by gavage in Wistar strain	GS0119-017 Terata NOEL = 31.2 mg/kg Terata LEL = 62.5 mg/kg (microphthalmia and increased fetal mortality; reduced fetal weight) Dosage levels = 15.6, 31.2, 62.5 and 125 mg/kg by gavage, Wistar rat	GS0119-017 NOEL = 169 mg/kg LEL = 298 mg/kg (weight decrease in fetuses). No dose related incidences of anomalies or malformations Dosage levels = 0 - 500 mg/kg in diet in Wistar rats
EPA	Accession	Acc.# 248563-A	749749-4 MRID 00115674	MRID 00096514	CS0119-018 MRID 00078620	œ0119-016	GS0119-017	GS0119-017
	1	Technical 99.1% Pure		Benanyl 99-99.2% pure	Benomy1	Benamyl	Benomyl	Bencmyl
Tox Chem No. Benomyl 75A	Stude / to the desired	Terationy - rat; Haskell Labs.; renort #587-80.	E.I. DuPont de Nemours;	2 Year feeding - mouse; Dupont Haskell Lab; 20-80; 1/26/82	Teratology - rat; Sherman et al.; 1975	Teratology - rat; Midwest Res. Inst.; #68-02-2982 1979	Teratology - rat; Health Effects Res. Lab; US. EPA; 1/11/80	Teratology - rat; Health Effects Res. Lab; US EPA ; 1/11/80

ORE Grade/ Doc. No.	003728 Supplementary 004689 Minimum when combined with	28 mum 79 - 67	003744 acceptable 004679 –	003744 acceptable 004679 –	003744 acceptable 004679 -	44	able -	003744 MV acceptable C5
Ourrent Date TOK ODE Category Doc	003728 Supple 004689 Minima combin 003042	003728 minimm 004679	003744 accepta 004679	003744 accept: 004679	003744 accept. 004679	003744	003744 accepts 004679	003744 accepts 004679
Results: LDsO, LCsO, PIS, NOEL, LEL	Unilateral microphthalmia at 10 mg/kg/day (2 animals) NOEL = 30 mg/kg LEL = 62.5 mg/kg (embryotoxicity) Dosage levels - 0, 3, 10, 62.5, 125 mg/kg/day by gavage in ChR-CD rats	No evidence of delayed neurotoxicity was found NOEL other neurotox, signs =2500mg/kg Dosage levels = 500, 2500,5000 mg/kg	GS0119-003 Mutagenic - significant dose related increase in micronuclei in bone marrow from femor bones at all doses bosage levels = 250, 500, 1000 mg/kg	GS0119-002 Dose related increase in mutation frequency at TK locus of L5178Y cells, in vitro - weak mutagen with and without activation	Weakly positive for sister chromatid exchange, levels tested with activ. 375-150 ug/ml; without activ. 625-10 ug/ml	Not mutagenic in TA 1537, 1535, 98 and 100 up to dosage levels of 250 mg/plate	GS0119-001 Mutagenic for strains TA 1537 and 98 with activation (Dose levels = 100 - 10,000 ug)	Not mutagenic at the HGPKT locus with or without activation,
EPA Accession No.	Acc.# 256575 CS0119-009	Acc.# 241930 CS0119-007	œ0119-003	CS0119-002	CS0119-004		CS0119-001	MRID 00038808
Material	Bencmy1	Denomyl in corn oil (99% Tech.)	Bencinyl	Benomyl (99% a.i.)	Bencmyl (99% a.i.)	Benomyl	Benomyî (99.68 a.i.)	Benomyl MRID (99.9-100% a.i) 00038808
Tox Chem No. benomyl 75A Study/Lab/Study #/Date	Teratology – rat; Haskell Lab; #649–80; 1980	Neurotoxicity - chicken; Bencmyl in corn IRDC;125-039; 10/8/79 oil (99% Tech.)	Mutagenic- micronucleus test - mice; SRI Int., (Kirkhart; 1980);LSU- 7553-19;2/12/80	Mutagenic - L5178Y TK+ (mouse lymphoma); SRI Int.;LSU-7558; Dec, 80	Mutagenic - SCE- chinese hamster ovary; SRI Int.; LSU-5778; Aug, 80	Mutagenic - microorgan- isms; Donvan and Krahn; 1981	Mutagenic - S.Typhim. Haskell; 560-80; 8/2/80	Mutagenic - ovary cells - chinese hamster;

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OORE Grade/ Doc. No.	003744 acceptable 004679 -	003744	003744 minimum 004679	000720	supplementary 004679 –	incomplete	004679 -		_
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Results: LDso, LCso, PIS, NOEL, LEL	GS0119-005 Not a mutagen when tested for DNA repair using mouse hepatocyte cultures	Dosage levels=0, 50, 100, 200 mg/kg given by gavage NOEL = 50 mg/kg LEL = 100 mg/kg (supra occipital scars, subnormal vertebral centrum, supernumerary ribs, cleft palate)	NOEL = 0.2 mg/L (7.5 mg/kg) LEL = 0.82 mg/L (reduction of spermatogenic activity)(33 mg/kg)ChR-CD LC50 > 0.82 doses tested: 0, 0.02, 0.12, 0.2, 0.82 mg/l	Systemic NOEL > 200 mg/kg/day for spermatogenic effects LEL = 3400 mg/kg/day (4/6 deaths) levels tested; 200, 3400 mg/kg/day in ChR-CD strain	LD ₅₀ > 9590 mg/kg Levels tested: 500, 2250, 3400, 3600, 7500, 9590 mg/kg in ChR-CD rats 1 rat/dose, all had testicular alterations	GS0119-008 increased micronucleus formation	NOEL = 500 mg/kg benomyl LEL = 1000 mg/kg MBC NOEL = 50 mg/kg MBC	for serum concentration NOEL = 8 ug/kg MBC LEL = 11.5 ug/kg " no chromosome breaks in vivo in hamster bone marrow at up to 1000mg/kg	
Accession No.	GS0119-005		MRID 00097281	MRID 00097601	MRID 00097601	800-611050			_
Material	Benonyl	Benomyl	Benomyl 50% WP (50%a.i)	Benomyl 1991 (% unspecified)	Benomyl 1991 (% unspecified)	Benomyl	MBC		
Study/Lab/Study #/Date	Mutagenic - mouse - DNA repair; Haskell; 741-81; 10/20/81 (Tong, 1981)	Teratology - mice; Kavlock et al; 1982 (Tox & Appl Pharm, 62: 44-54; 1982)	Acute imhalation - rat; Haskell Lab; #95-69; 4/24/69	14-day intubation — rats Haskell labs; 100-66; 7/15/66	Acute oral - rats; Haskell labs; 100-66; 7/15/66	Mutagenic micronucleus -	mouse; J.P.Seiler; 1976		page 5/8

18	•				Katha Variation			2.3
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CORE Grade/ Doc. No.	supplementary 004679 –	minim.m 004679 -	unacceptable 004679 -	supplementary RS IXC #	acceptable	# # # # # # # # # # # # # # # # # # #	minimum 004679 -	
TOX		Ħ					111	
Results: LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL	No deaths, 1 rat/dose at 200,450, 670 and 1000 mg/kg. There was a dose response for decr. rel. testes weight. Tubular degeneration and necrosis of the testes was present at 670 and 1000mg/kg in ChR-CD rats	LC ₅₀ > 1.65 mg/l (HDT no deaths) NOEL = .65 mg/l (32 mg/kg) LEL 1.65 mg/l (82 mg/kg)based on reducedspermatogenic activity at 14 d (notpresent at 28 days)	The major urinary metabolites of benomyl, after 12 days of 2500 pgm in the diet followed by a 7.7 mg oral dose of benomyl-214C, are conjugates of 5-OH-MBC.	1) mouse, rabbit and sheep had similar metabolite distribution 2) oral and intraperitoneal routes were similar as excreted by 96 hr (majority in urine) 4) no parent in urine or feces.	GS0119-014 Bencmyl was absorbed in a nonlinear dose and duration related manner. * absorbed ranged form .031 (after high dose of 100 mg a.i.) to 3.518 (after low dose of 0.1 mg a.i.) after 10 hours.	GS0119-013 Benomyl and MBC induced genetic segregation in a heterozygous green diploid strain of A. nidulans.	PIS 28 7.3 0	
Accession No.	MRID 00066779	MRID 00097275	MRID 00066776 Acc.# 091561-F	MRID 00036818	GS0119-014	GS0119-013	MRID 00084579	
Material	Bencray1	Вепстуј WP 50% 50 % а.i.	Белсту 1	benomyl	benomyl 50%WP 50% a.i.	benomy1	Benlate Df 75 % a.i.	***
Study/Lab/Study #/Date	Acute oral – rats; Haskell labs; 179–65; 12/15/65	Acute inhalation - dogs; Hazelton labs; HLR#192- 69; 7/14/69	Metabolism - rat (1); DuPont; #?; 1968?	Metabolism; Douch, PGC; Xenobiotica; 3(6):367- 380	Dermal absorption - rat; duPont; #?; 3/9/79	Mutation - A. nidulans; Kappas, et al; Mutat. Res.; 26(1) 1974, 17-27	Primary eye irritation - rabbits; Haskell labs; 179-81;4/6/81	۵, ۶

	Study/Lab/Study #/Date	Material	No.	IDEA ICEA PIS NOEL IEI	Category	Ourt of ade/	
	90-day feeding-dog Haskell Lab.; #283-70; 1970	50% WP (53% tech) MBC	130	Systemic NOEL = 500 ppm (14 mg/kg/d) LEL = 1500 ppm (41 mg/kg/d) based on incr.alk. phos., chol, SGPT and minimal microscopic alterations in the liver (1/4 males and females) and testes (1/4 males) levels tested: 0, 100, 500, 2500/1500 ppm a.i.		minimum 004679	
	2 Year feeding/onco-rat; Haskell Lab.; 195-72; 1972	50 or 70 % ai (53 or 72.2 % tech.) MBC	Acc. # 232870-C 232871 MRID 00088333	Systemic NOEL = 500 ppm LEL = 5000 ppm based on decr. wt. gain, decr. HCT, HCB and RCB in females; incr. pericholangitis in males and females. Onco. NOEL > 10,000 ppm (HDT) Doses tested: 0, 100, 500, 5000, 2500/10000 ppm a.i.		minimun 004679 –	
	2 Yoar feeding - dog; Haskell Lab.; 195-72; 1972	50 or 70 % ai (53 or 72.2 % tech.) MBC	Acc.# 232870-C 232871 MRID 00088333	Systemic NOEL = 100 ppm LEL = 500 ppm based on biochem, and histological alterations indicating liver damage; levels tested: 0, 100, 500, 1500/2500 ppm a.i.		minimm 004679 –	
	3 generation repro – rat Haskell Lab.; 195–72; 1972	50 or 70 % ai (53 or 72.2 % tech.) MBC	Acc.# 232870-C 232871 MRID 00088333	NOEL = 500 ppm LEL = 5000 based on decr. pup weight at weaning		minimum 004679 -	
	2 Year Onco - mice; Woods et al, 1982 Haskell Lab.	МВС	CS0119-010	NOEL = 500 ppm LEL = 1500 ppm based on increased incidence of hepatocellular carcincmas, lymphoid depletion of the thymus in males and females. Hepatotoxic lesions were only present in the males. Dose levels: 0, 500, 1500, 7500(females), 7500 for 15 mo, then 3750 (males) ppm in CD-1 strain.		Mei 00467	
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Study/Lab/Study #/Date	Material	No.	IDEO, ICEO, PIS, NOEL, LEL	Category	Doc. No.
96 Week onco - mice; Kramer and Weigand et et al., 1982.	MBC	GS0119-011	Not oncogenic at 5000 ppm NOEL = 300 ppm LEL = 5000 ppm based on increased abs. and rel. liver weight, marked hepatocellular necrosis and cellular alterations indicative of toxicity. Dose levels: 0, 100, 300, 1000 (increased to 5000 at 8 weeks) ppm in NWRKE(SPF 71)strain		WHO review
80 Week onco - Mice Beans, 1976	MBC	CS0119-012	NOEL = 300 ppm LEL = 5000 ppm based on increased incidence of neoplastic nodules of the liver in females, and hepatoblastomas in males, altered rel. liver weight in males and females. Doses tested 0, 150, 300 and 1000 (increased to 5000 at 8 wks) in SPF Swiss mice.		WHO review
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	E. Greenhouse - Food Crop	[R]	YES	;			
	Greenhouse - Nonfood	[R]	YES	<u> </u>			
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[X] A. Terrestrial - Food Crop [X] B. Terrestrial - Nonfood [] C. Aquatic - Food Crop	[R]	YES	
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D. Aquatic - Nonfood	(R)		
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: [X] G.	Forestry	[R]	YES	
·	. Domestic Outdoor	[R]	YES	:
· · · · · · · · · · · · · · · · · · ·	. Indoor	[R]		
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DATA REQUIREMENT	r FOOTNOTES.			

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	XICOLOGY - SUBCHRONIC TESTING		::::::::::::::::::::::::::::::::::::::	**********
- 82	2-1 - 90-day feeding - rodent, r	non-rodent		
	; Carbamic acid,(1-((butylamin			
	1 for this requirement::DATED			
	Use	Guideline		
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: (X) : (X)	A. Terrestrial - Food Crop B. Terrestrial - Nonfood	[R]	YES	
: A	C. Aquatic - Food Crop D. Aquatic - Nonfood	[R]		
; (X) ; (X)	E. Greenhouse - Food Crop F. Greenhouse - Nonfood	[R]	YE	
: (X)	G. Forestry H. Domestic Outdoor			
: 🔘	I. Indoor	[R]		
:STATUS OF DA	TA REQUIREMENTS		• • • • • • • • • • • • •	•
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:CITATIONS:	(S= Fully satisfactory P=			
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	85 (P) (dog)			:
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ŧ٨.	I.=Benomy)	l;	Carbamic act	id,(1-((buty)	lamino)	carbonyl)-l	H-benzimidazo	le-2-yl),	:
	methyl	est	er			_			
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:	(X)	A.	Terrestrial	- Food Crop)	[CR]	yes		:
:	ĺΧ̈́	В.	Terrestrial	L - Nonfood		[CR]	yes		:
•	()	C.	Aquatic - E	Food Crop		[CR]			:
•	iri	D.	Aquatic - N	ionfood		[CR]			•
:	र्ज	£.	Greenhouse	- Food Crop		[CR]	ves		:
:	} \$ {		Greenhouse			[CR]	yes		:
•	<u>(A)</u>			- Montoon			· · · · · · · · · · · · · · · · · · ·		•
:	[X]		Forestry			[CR]	yes		:
:	(<u>X</u>)		Domestic Ou	itaoor		[CR]	yes		:
:	(_)	I.	Indoor			[CR]			:
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	TOXICOLOGY - SUBCHRONIC TESTING 82-3 - 90-day dermal	:		
:A.I.=Benomy	yl; Carbamic acid, (1-((butylami	no)carbonyl)-lH-	-benzimidazol	e-2-yl), :
	l ester			
:PAGE 1 of	1 for this requirement::DATED	/::Superd	cedes page da	ted/;
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: (X) : (X)	A. Terrestrial - Food Crop B. Terrestrial - Nonfood	[CR]	no	·
: (<u>X</u>)	C. Aquatic - Food Crop	[CR] [CR]	no	 :
: ;;	D. Aquatic - Nonfood	(CR)		:
	E. Greenhouse - Food Crop	[CR]	no	:
: (X)	F. Greenhouse - Nonfood	[CR]	no	:
: (<u>X</u>)	G. Forestry	[CR]	no	:
İ	H. Domestic Outdoor	[CR]	no	
: A	I. Indoor	[CR]		
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-11- \$158.135 - TOXICOLOGY - SUBCHRONIC TESTING: - 82-4 - 90-day inhalation - rat :A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), :
\$158.135 - TOXICOLOGY - SUBCHRONIC TESTING: - 82-4 - 90-day inhalation - rat
- 82-4 - 90-day inhalation - rat
: methyl ester
:PAGE 1 of 1 for this requirement::DATED/:Supercedes page dated/:
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: [X] A. Terrestrial - Food Crop [CR] yes :
: [X] B. Terrestrial - Nonfood [CR] yes : : [] C. Aquatic - Food Crop [CR] :
: [] D. Aquatic - Pood Crop (CR) :
: [X] E. Greenhouse - Food Crop [CR] yes : [X] F. Greenhouse - Nonfood [CR] yes :
: [X] G. Forestry [CR] <u>yes</u> : : [X] H. Domestic Outdoor [CR] <u>yes</u> :
: [] I. Indoor [CR]
:STATUS OF DATA REQUIREMENTS :
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	ICOLOGY - SUBCHRONIC TESTING			
	5 - 90-day neurotoxicity - he			
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	Carbamic acid, (1-((butylamin	no)carbonyl)-lH-d	œnzimidazole	-2-y1), :
: methyles				
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•	A. Terrestrial - Food Crop	[CR]	no	
: (X) i	B. Terrestrial - Nonfood	[CR]	no	;
	C. Aquatic - Food Crop	[CR]		
	D. Aquatic - Nonfood	[CR]		:
	E. Greenhouse - Food Crop	[CR]	no	:
: (X) 1	F. Greenhouse - Nonfood	(CR)	no	
: (X)	G. Forestry	[CR]	no	
: (X) 1	H. Domestic Outdoor	(CR)	no	:
: 🗍 :	I. Indoor	(CR)		:

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\$158.135 - TO	OXICOLOGY - CHRONIC TESTING:			
- 83	3-1 - Chronic feeding - (2 spp.)	- rodent, non-	rodent	
:A.I.=Benomy	l; Carbamic acid, (1-((butylamin	o)carbonv1)-1H-	benzimidazol	e-2-vl), :
: methyl	ester	•		•
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: (X)	A. Terrestrial - Food Crop	[R]		•
(X)	B. Terrestrial - Nonfood	[CR]		:
: ∺	C. Aquatic - Food Crop	[R]		:
: 8	D. Aquatic - Nonfood	[CR]		
	E. Greenhouse - Food Crop	[R]	YES	 :
: [X] : [X]	F. Greenhouse - Nonfood	[CR]	165	
		[CR]	-	:
: (X) : (X)	G. Forestry H. Domestic Outdoor	[CR]		
	I. Indoor	[CR]	***************************************	:
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:	20.4 (m) (:
	284 (P) (rat)			:
: 000973	305, 00081913, 00097318, 0009732	(F)	(dog)	,
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	XICOLOGY - CHRONIC TESTING : -2 - Oncogenicity study - (2 s	nn I - rat mou		
	-2 - Grogenicity study - (2 a			
	; Carbamic acid, (1-((butylami			
: methyl		,		- 1-//
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: (X) : (X)	A. Terrestrial - Food Crop B. Terrestrial - Nonfood	[R] [CR]	YES	:
	C. Aquatic - Food Crop	[R]		;
: H	D. Aquatic - Nonfood	[CR]	-	
: <u>(X</u> 1	E. Greenhouse - Food Crop	[R]	YES	
: (X)	F. Greenhouse - Nonfood	[CR]		:
: [X]	G. Forestry	[CR]		:
: (<u>X</u>)	H. Domestic Outdoor	[CR]		:
: U	I. Indoor	[CR]		:

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: 000972	84 (P) (rat) ·			:
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\$158.135	- TOXICOLOGY - CHRONIC TESTING:			
	- 83-3 - Teratogenicity - (2 spp.			
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	nomyl; Carbamic acid,(1-((butylam	ino)carbonyl)-lH	-benzimidazol	e-2-y1), :
	thyl ester			
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: (X)	A. Terrestrial - Food Crop	[R]	YES	
i ixi	B. Terrestrial - Nonfood	[CR]		
. (C. Aquatic - Food Crop	[R]		:
i	D. Aquatic - Nonfood	(CR)		:
: (X)	E. Greenhouse - Food Crop	(R)	YES	:
: (X)	F. Greenhouse - Nonfood "	(CR)		:
: [X]	G. Forestry	(CR)		:
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: ()	I. Indoor	[CR]		:
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	0115674, 00126522 (P) (rat)			i
	50119-009 (P) (rat)			•
: 68	50119-017 (P) (mouse)			:
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\$158.135			NIC TESTING:					
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: (<u>X</u>		Terrestrial		[R] [CR]		<u> </u>	-	
: [<u>X</u>]		Aquatic - F		[R]				:
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: ₩		Greenhouse		[CR]		-	-	:
· ix		Forestry	10112000	[CR]				 ;
र्ध्य		Domestic Ou	tdoor	[CR]				 ;
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: [X]	A. Terrestria	l - Food Crop	i	[R]	YES			
: (<u>X</u>)	B. Terrestria	l - Nonfood	ĺ	CR]				
: ()	C. Aquatic - 1		1	[R]	-			
: 🗇	D. Aquatic - 1		ĺ	CR]				
: (X) : (X) : (X)	E. Greenhouse			[R]	YES			
: (<u>X</u>)	F. Greenhouse	Nonfood		[CR]				
: <u>[X]</u>	G. Forestry			(CR)		-		
	H. Domestic O	utdoor	•	CR]				
: U	I. Indoor		•	[CR]				
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	KICOLOGY - MUTAGENICITY TESTING	; :		
- 84-	 4 - Other genotoxic effects 			
				:::::::::::::::::::::::::::::::::::::::
A.I.=Benomyl;	Carbamic acid, (1-((butylamir	no)carbonyl)-lH-	-benzimidazol	e-2-yl),
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	A. Terrestrial - Food Crop	(R)	_YES	
122	B. Terrestrial - Nonfood	[CR]		
	C. Aquatic - Food Crop	[R]		
	D. Aquatic - Nonfood	[CR]		
(X)	E. Greenhouse - Food Crop	[R]	YES	
(<u>X</u>)	F. Greenhouse - Nonfood	(CR)		
	G. Forestry	[CR]		
(<u>X</u>)	H. Domestic Outdoor	[CR]		
(_)	I. Indoor	[CR]	-	

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158.135 - TO	OXICOLOGY - SPECIAL TESTING:			
- 85	5-1 - General metabolism			
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	l; Carbamic acid, (1-((butylami			
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(X)	A. Terrestrial - Food Crop	(R)	YES	
	B. Terrestrial - Nonfood		165	
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¦늬	C. Aquatic - Food Crop	[R]		
<u> </u>	D. Aquatic - Nonfood	[CR]		
ίχι	E. Greenhouse - Food Crop	[R]	YES	
Ю	F. Greenhouse - Nonfood	[CR]		
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:A.	<pre>I.=Benomyl; methyl es</pre>		id,(l-((butylam	ino)carbonyl)-1	I-benzimidazol	e-2-yl), :
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4/85
\$158.135 - TOXICOLOGY

*A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), : methyl ester

*PAGE _2 of _ for this requirement::DATED _/ _::Supercedes page dated _/ _:
DISCUSSION OF DATA:

BENOMYL #75A

604679

study type			study type		
doc 1			mpp.mo: 00.4		
ACUTE ORAL STUDIES			TERATOLOGY	_	e 1.
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179-81	7	37	197-12 -140	P	-5
SKIN IRRITATION TEST-RABBIT		۱ د			
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	P	40			
84-69 (Guinea pig) SKIN SENSITIZATION	þ	40			
		1. 0			
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cont.					

STUDY TYPE: Acute oral LD50 - Rats

TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 174-65

FICHE/MASTER: 00066779

MR NO.: 581

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial

Medicine, Wilmington, Del.

AUTHORS: G.M.Zwicker, H.Sherman

DATE REPORT SUBMITTED: December 15, 1965

TEST MATERIAL: Benomyl; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic

acid, methyl ester

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-lH-benzimidazol-

2-yl)-methyl ester

INT-1991 NB- 5409-91

DPX-3866

N.B. 8084-166B

MATERIAL AND METHODS: Young ChR-CD rats were given single doses of test compound in peanut oil, by stomach tube. The dosing schedule was as follows:

Dose (mg/kg)	14 day surv./Treate	ed Rel. Testis Wt (gm)
200	1/1	0.86
450	1/1	0.86
670	1/1	0.77
1000	1/1	0.79

The survivors were necropsied after 14 days and the testes weighed and compared to the final body weight. They were also evaluated for visual and microscopic alterations.

RESULTS: There was no apparent change in body weight. There may have been an absolute and relative testicular weight decrease however there were too few animals to confirm this. Both testes were small and soft in the 670 mg/kg treated rats. Up to 10 % of the seminiferous tubules had degeneration and necrosis of the germinal epithelium, absence of mature sperm in the tubules, and multinucleated germinal giant cells (670 and 1000 mg/kg treated animals).

DISCUSSION: There was definite evidence of macroscopic and microscopic testicular dammage at the two high doses tested. Too few animals were tested to determine the significance of the target organ weight changes. Although no other toxic signs were noted, the report did not say whether they were looked for. The report did not give the composition of the test compound (formulation or technical, and \$a.i.).

CONCLUSIONS: The testis is a target organ with tubular degeneration and necrosis.

CORE CLASSIFICATION - supplementary

Reviewed by M.P.Copley, D.V.M. Tox. Br. 9/12/85

STUDY TYPE: Acute oral LD50 - Rats

TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 100-66

FICHE/MASTER: 00097601

MR NO.: 581

SPONSOR: E. I. du Pont de Nemours and Company

Haskell Lab. for Toxicology and Industrial STUDIES PERFORMED AT:

Medicine, Wilmington, Del.

AUTHORS: H.Sherman, W.C.Krauss

DATE REPORT SUBMITTED: July 15, 1966

TEST MATERIAL: Benomyl; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic

acid, methyl ester

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-lH-benzimidazol-

2-yl)-methyl ester

INT-1991

NB- 5409-91 DPX-3866

N.B. 8084-166B

MATERIAL AND METHODS: Young adult male ChR-CD rats were given single doses of test compound in a 20-22.5 % suspension of peanut oil, by

stomach tube. The dosing schedule was as follows: % tubules affected Dose (mg/kg) Dead/Treated_ Rel. Testis Wt (gm) 0.83 1500 0/1 < 10 > 70 2250 0/1 1.29 > 70 3400 0/1 0.69 < 10 0.71 0/1 5000 0.48 > 70 7500 0/1 0/1 0.42 <u>> 10 < 70 </u> 9590

The survivors were necropsied after 14 days and the testes weighed and compared to the final body weight. They were also evaluated for visual and microscopic alterations.

RESULTS: There were no deaths or change in body weight. Although there appeared to be a 50 % decrease in absolute and relative testicular weight, there were too few animals to confirm this. Testes from rats given greater than 1500 mg/kg of material were small. See the above table for percent of seminiferous tubules with degenerated germinal epithelium, multinucleated giant cells and reduced to no sperm. Rats treated with 3400 mg/kg and greater also had reduced or no sperm in the epididymis.

DISCUSSION: There is definite evidence of testicular damage, both macroscopic and microscopic, at all doses tested. Too few animals were tested to determine the level of significance of the target organ weight changes. Although no other toxic signs were noted,

the report does not say whether they were looked for. The report also does not give the composition of the test compound (formulation or technical, and % a.i.).

CONCLUSIONS: There were too few animals/dose to determine $\rm LD_{50}$ (no death at 9590 mg/kg - 1 rat). The testis is a target organ with tubular degeneration and necrosis.

CORE CLASSIFICATION - supplementary

Reviewed by M.P.Copley, D.V.M.

Tox. Br.

9/12/85

(Original review by L.B.Dale, October 23, 1968)

0,04679

STUDY TYPE: Acute oral LD50 - Rats

TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 421-80

FICHE/MASTER: 64819

MR NO.: 581-867 ACC.: 243043

SPONSOR: L. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial

Medicine, Wilmington, Del.

AUTHORS: J.A. Hall, G.L. Kennedy

DATE REPORT SUBMITTED: May 23, 1980

TEST MATERIAL: Benlate®, (75 % a.i.); active ingredient: Benomyl;

1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid,

methyl ester

SYNONYMS: Benomyl Dry Flowable concentrate

Benlate DF Fungicide

a.i.: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidazol-

2-yl)-methyl ester

INT-1991

NB- 5409-91 DPX-3866

N.B. 8084-166B

Original review:

"Procedure: A group of (fasted) # 5M (ave.wt. 226 gm) #, 5F (ave.wt. 154 gm) # Chk-CD rats received oral application of the test substance by intubation. The substance used was "Benlate DF Fungicide" suspended (30% suspension) # in corn oil at a dosage rate of 5000 mg/k. The animals were observed for 14 days. Survivors were sacrificed at the termination of the study; all animals were necropsied.

<u>Results</u>: No mortalities. Symptoms included stained face, stained an wet perineal area, chromodacryorrhea and weight local Necropsies revealed testes slightly-small to small, soft, grey with white subcapsular streaks and foci; livers - slightly heavy; lungs - pale red with grey foci throughout. LD_{50} is greater than 5000 mg/kg.

Toxicity Category: IV - CAUTION "

Original reviewer: M.L.Quafe (3/25/70)

Core Classification: minimum

<u>CONCLUSION</u>: LD₅₀ > 5000 mg/kg (75 % a.i.)

The testes appears to be a target organ.

Review evaluated by M.P.Copley, D.V.M.
Tox. Br.

9/12/85

()* Facts from study inserted into original review by M.P.Copley for completeness.

STUDY TYPE: Acute oral LD50 - Rats

TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 17-69

· FICHE/MASTER: 97277

MR NO.: 581

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial Medicine, Wilmington, Del.

AUTLORS: H.Sherman, J.A.Zapp

DATE REPORT SUBMITTED: Jan. 22, 1969

TEST MATERIAL: Benlate® (53 % tech, % a.i. not

given); active ingredient: Benomyl; 1- (Butylcarbamoyl)-2-benzimidazolecarbamic acid,

methyl ester

SYNONYMS: a.i.: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-

benzimidazol-2-yl)-methyl ester

INT-1991 NB- 6299-77

DPX-3866 N.B. 8084-166B Haskell No. 5833

MATERIAL AND METHODS: A group of 10 male* (ave. wt. 254 gm) and 10 female (ave. wt. 181 gm) ChR-CD rats were fasted then treated with a 20 or 30 % suspension of compound in peanut oil at a dosage rate of 10,000 mg/kg**. The animals were observed for 14-16 days.

RESULTS: There were no mortalities. No other data was given.

CONCLUSION: LD50 is greater than 10,000 mg/kg (53 % tech.)

Core Classification: Minimum

Toxicity Category: IV

Reviewed by M.P.Copley, D.V.M. Tox. Br. 9/12/85

63

** Based on active ingredient.

^{*}Consisted of 2 groups of males with 5 each treated at different times. Female group of 10 was treated at ont time.

STUDY TYPE: Acute oral LD₅₀ - Rats TO

TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 17-69

FICHE/MASTER: 97277

MR NO.: 581

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial

Medicine, Wilmington, Del.

AUTHORS: H.Sherman, J.A.Zapp

DATE REPORT SUBMITTED: Jan. 22, 1969

TEST MATERIAL: Benomyl technical; \$ a.i. not given, active

ingredient: Benomyl; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester

SYNONYMS: a.i.: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-

benzimidazol-2-yl)-methyl ester

INT-1991

NB- 6275-176

DPX-3866

N.B. 8084-166B

Haskell No. 5834

MATERIAL AND METHODS: A group of 10 male* (ave. wt. 245 gm) and 10 female (ave. wt. 180 gm) ChR-CD rats were fasted then treated with a 20 or 30 % aqueous suspension of compound at a dosage rate of 10,000 mg/kg**. The animals were observed for 14-16 days.

RESULTS: There were no mortalities. No other data was given.

CONCLUSION: LD50 is greater than 10,000 mg/kg (tech.)

Core Classification: Minimum

Toxicity Category: IV

Reviewed by M.P.Copley, D.V.M. Tox. Br. 9/12/85

** based on active ingredient.

^{*}Consisted of 2 groups of males with 5 each treated at different ti-Female group of 10 was treated at ont time.

STUDY TYPE: Acute dermal LD50 - Rabbits

TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 554-80

FICHE/MASTER: 000648:

MR NO .: 0581-867

ACC. No.: 243043

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES FERFORMED AT: Haskell Lab. for Toxicology and Industrial

Medicine, Wilmington, Del.

AUTHORS: O.L.Dashiell, P.Ashley

DATE_REPORT SUBMITTED: July 23, 1980

TEST MATERIAL: Benlates, (75 % a.i.); active ingredient: Benomyl;

1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid,

methyl ester

SYNONYMS: Benomyl Dry Flowable concentrate

a.i.: Carbamic Acid, (1-((Butylamino)-carbonyl)-lH-be...imidazol-

2-yl)-methyl ester

INT-1991 NB- 5409-91 DPX-3866 N.B. 8084-166B

MATIRIAL AND METHODS: Five male (ave. wt. 2848 gm) and five female (ave. wt. 2071 gm) adult albino rabbits, New Zealand White strain, were clipped over the back and trunk and plastic collars attached. Two thousand mg/kg of test substance (moistened with physiological saline) was applied to abraded skin on each rabbit and covered with gauze pads and wrapped with plastic wrap and adhesive bandage. After 24 hr the material and wrapping were removed and the treated skin wiped dry. The animals were observed and weighed during a 14 day period then sacrificed. Only two rabbits per sex were necropsiat that time.

RESULTS: There were no deaths. Although there was sporatic weight loss in the males (from 1-14 days) and females (1-7 days) there was an average 14 day weight gain of 11 % for the males and 21 % for the females. Moderate to moderate-severe skin irritation was present all animals the day after dosing. All rabbits had returned to normal by day 14, except 1 male and 1 female. No compound related abnormalities were observed at necropsy.

CONCLUSIONS: LD50 > 2000 mg/kg (75% a.i.)

TOXICITY CATEGORY: III

CORE CLASSIFICATION: Guideline

Reviewed by M.P.Copley, D.V.M.

Tox. Br. 9/12/85

(Original review by Sherell Sterling, 11/19/

STUDY TYPE: Acute dermal LD50 - Rabbits

TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 201-216

FICHE/MASTER: 009760

MR NO.: 581-239

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Hazleton Labs., Inc., Falls Church, Va.

AUTHORS: W.M. Busey

DATE REPORT SUBMITTED: June 21, 1968

TEST MATERIAL: Benomyl; 50 % wettable powder; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester

SYNONYMS:

a.i.:Carbamic Acid, (1-((Butylamino)-carbonyl)-lH-benzimidazol 2-yl)-methyl ester

INT-1991 NB- 5409-91 DPX-3866 N.B. 8084-166B

MATERIAL AND METHODS: Abdomens of 4 rabbits (2.3-2.9 kg) per dose (2 abraded, 2 intact skin) were exposed for 24 hr to 464, 1000, 2150, 4640 mg/kg of test material covered with an occlusive dressin One rabbit each was exposed to 3430 and 10,000 mg/kg, also with an occlusive dressing for 24 hr. After treatment, the skin was washed with water to remove the remaining compound. Animals were given food and water ad libitum and observed daily for 14 days for death and toxicity. Body weights were taken before and at termination of the study. All survivors were necropsied and the testes were examined microscopically for several rabbits.

RESULTS: There were no deaths. One animal (out of four) in each of the first four treatment groups developed non-treatment related enteric problems and anorexia. Dermal irritation at 464, 1000, 215, 4640 mg/kg was slight to moderate (slight erythema and moderat edema), subsiding by day 4 or 5. There was also slight desquamatio on days 4 and 5. At 3430 and 10,000 mg/kg, there was marked erythe lasting 7 days and slight edema lasting 2 days. There was also slight desquamation observed by day 5. There were no treatment related lesions observed at necropsy or histopathologic changes in the testes.

CONCLUSIONS: LD₅₀ > 4640 mg/kg (50% WP)

TOXICITY CATEGORY: III

CORE CLASSIFICATION: minimum

Reviewed by M.P.Copley, D.V.M.
Tox. Br.
9/12/85

Originally reviewed by L.B.Dale, 10/23/68:
The original review listed the LD₅₀ as > 10,000 mg/kg however M.
Copley disagrees because only 1 rabbit was tested at 10,000 mg/kg.

مان

STUDY TYPE: Acute inhalation LC50 - Rats

TOX. CHEM. NO.: 7

HAZELTON PROJECT NUMBER: 201-220

FICHE/MASTER: 000:

MRO NO .: 1126

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Hazelton Lab., Inc., Falls Church Va.

AUTHOR: W.M. Busey

DATE REPORT SUBMITTED: October 18, 1968

TEST MATERIAL: Benomyl; 50 % wettable powder; l-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester; 50 %

SYNONYMS: Fungicide 1991

a.i.: Carbamic Acid, (1-((Butylamino)-carbonyl)-lH-benzimida:

2-yl)-methyl ester

INT-1991 NB- 5409-91 DPX-3866

N.B. 8084-166B

MATERIAL AND METHODS: Four groups of 6 albino rats (ave. wt. 293 gm) were exposed in an inhalation chamber to Benomyl for 4 hr at the following analytical concentrations:

J.1.5 1 0 1 2 1								
_mg/L	dead/treated	Aspermatogenesis (severity	/) #/survivor:					
0	1/6		0/5					
U.27	0/6	slight to moderate	1/6					
1.39	0/6	slight	1/6					
4.01	0/6	severe	2/6					

The chamber was a 1000 L plexiglass and stainless steel container with aerosolization by means of a constant airflow generator or a Devolbiss powder blower. The analytical concentration was determined by gravimetric analysis at least twice during the exposure. The animals were individually housed in the exposure chamber, then by group for the duration of the 14 day observation period. The rat were observed frequently during the exposure period for toxic signand death, then daily for the duration of the study. Necropsies performed on all animals either at death or at the 14 day sacrificoss lesions, lungs, trachea, liver, kidneys and testes were examistologically. Other organs were saved in 10% buffered formalic

RESULTS: Mortality - There was 1 death in the control group on d due to a preexisting condition.

Observations - During exposure the 1.39 mg/L group animals were covered with a white powder within 15 min. After 45 minutes breathing became progressively more labored and shallow with gas; There was excessive lacrimation and salivation. The rats recover shortly after cessation of exposure. The 4.01 mg/L rats were not visable due to the high concentration of compound in the chamber. After 2 hr the aerosol was reduced momentarily to visualize the

animals. They were inactive, gasping and heavily coated with dustries resumed normal activity and treathing patterns after removal from the chamber. All animals were normal from day 1 till the and of the study with the exception of 1 death in the control group (noted earlier).

Necropsy - Most animals had lung discolorations and spots of variables and sizes. There were no treatment related necropsy observations.

Pathology - The report noted a slight increase in severity and frequency of lung inflammatory lesions. There was also increased severity over controls of intrabronchiolar epithelial hyperplasia and perivascular infiltration of mononuclear cells and lymphocytem. There was also an increased severity over controls of tracheitis with inflammatory cell infiltration into the tracheal submucosa in the 4.01 mg/L. Aspermatogenesis was present in all treated group: (see table).

DISCUSSION: Although only 6 rats were used per group, the LD50 locan be estimated as greater than the high dose tested (no deaths a any treatment level). There is no mention of partical size. Sinco heavy dust was reported on the animals and cage some of the exposimal have been oral due to preening. The background of lung lesion in the controls due to using older animals, made it difficult to determine a treatment related response. The increased lung inflam lesions noted in the report's summary was not evident from the histopathology tables. The major target of this compound appeared to be the testes, characterized by aspermatogenesis. The report mentions a treatment related decrease in sperm production however, the average level of spermatogenic activity in those rats without aspermatogenesis was the same for all groups.

CONCLUSIONS: LC50 > 4.01 mg/L (50% a.i.). Aspermatogenesis is present at all levels tested (0.27 mg/L LDT).

TOXICITY CATEGORY: III

CORE CLASSIFICATION: Minimum

Reviewed by M.P.Copley, D.V.M. Tox. Br. 9/12/85

This study was originally reviewed by R.D.Coberly on 2/27/73.

-12-

STUDY TYPE: Acute inhalation LC50 - Rats

TOX. CHEM. NO.: 75A

HASKELL PROJECT NUMBER: 95-69

FICHE/MASTER: 00097281

MR NO.: 1192

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial

Medicine, Wilmington, Del.

AUTHOR: C.S.Hornberger

DATE REPORT SUBMITTED: April 24, 1969

TEST MATERIAL: Benomyl; 50 % wettable powder; 1-(Butylcarbamoyl)-

2-benzimidazolecarbamic acid, methyl ester; 50 % a.i.

(52.2% Tech.)

SYNONYMS: Benlate@ dust (Fungicide)

a.i.: Carbamic Acid, (1-((Butylamino)-carbonyl)-lH-benzimidazol-

2-yl)-methyl ester

INT-1991 NB- 5409-91

DPX-3866

N.B. 8084-166B

MATERIAL AND METHODS: Ten male Charles River Caesarean Derived rats (64-65 days old) were exposed for 4 hr to Benlate® dust - nose only. The dust was generated by exposing a falling stream of powder to a pneumatic jet. A small cyclone head was used to recycle particles less than 10 um to the generator. The dust then passed through a cylinder into which the heads of the rats projected. Particle size was determined by a cascade impactor. Actual concentrations, determined gravimetrically, were; 0, 0.02, 0.12, 0.20, and 0.82 mg/L. After exposure, the rats were observed for 7 (5 per group) or 14 days then necropsied. Body weight and testicular weights were noted. All testis and lungs were examined histologically Lympn nodes liver, spleen, and kidney were examined histologically in 1 rat per group per time period.

RESULTS:

There was no change in relative testicular weight due to the concentrations used. At 14 days 2/5 rats (0.82 mg/L) had decreased spermatogenesis. All other groups were normal except for one control rat who fell in the Benlate® dust for one hr and also had decreased spermatogenesis.

DISCUSSION: Oral exposure was minimized by using the nose only exposure. In the previous study at Hazelton (whole body) the low dose testicular effect may have been due in part to ingestion as the one control that fell into the dust also had decreased spermatogenesis.

-13-

004379

TOXICITY CATEGORY: II

CORE CLASSIFICATION: minimum

Reviewed by M.P.Copley, D.V.M. Tox. Br. 9/12/85

STUDY TYPE: Acute inhalation LC50 - Dogs

TOX. CHEM. NO.: 75A

HAZELTON PROJECT NUMBER: HLR-192-69*

FICHE/MASTER: 00097282

MR NO.: not given

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Hazelton Lab., Inc., Falls Church, Va.

AUTHOR: N.A.Littlefield

DATE REPORT SUBMITTED: 7/14/69

TEST MATERIAL: Benomyl; 50 % wettable powder; 1-(Butylcarbamoyl)-

2-benzimidazolecarbamic acid, methyl ester;

(information obtained from summary MRID # 00097275)

SYNONYMS: Benlate® Dust (Fungicide)

a.i.: Carbamic Acid, (1-(Butylamino)-carbonyl)-1H-benzimidazol-

2-yl)-methyl ester

INT-1991

NB- 5409-91

DPX-3866

N.B. 8084-166B

MATERIAL AND METHODS: Animals - Sexually mature male beagle dogs were assigned to 3 groups of 10 dogs each. Exposure - The animals were exposed for 4 hr to either; 1) Fungicide 1991 - Benlate Formulation at an actual concentration of 0.65 (LDT) or 1.65 (HDT) mg/l, or 2) filtered room air. They were in a 4000 liter stainless steel and glass chamber. The compound was aerosolized by a pneumatic-dust generator with a cyclone head. The air flow was 1500 1/min. for the LDT and 260 1/min for the HDT. Actual concentrations were determined by gravimetric analysis and particle sizes were determined with a cascade impactor. After exposure the dobs were rinsed in warm water and dried to decrease exposure by ingestion. Observations - Animals were observed for toxicity and death. They were weighed prior to treatment and weekly thereafter. The testes were palpated at unspecified intervals. Sacrifice - The dogs were necropsied after 14 days (5/group) or 28 days of observation. Testes from all animals were preserved in chilled Bouin's fluid. Other tissues were fixed in 10 % neutral buffered formalin. Trachea, lungs (all lobes), testes and liver were examined histologically for all animals. The following tissues were also fixed, however only one animal per group per time period had a complete histopathologic evaluation: brain, pituitary, thyroid glands, heart, gallbladder, spleen, kidney, eye, stomach, pancreas, small intestine, large intestine, lymph nodes, urinary bladder, bone, bone marrow, adrenal glands, thymus, cervical spinal cord, salivary gland, lacrimal glands, prostate gland, sciatic nerve, skeletal muscle, aorta, and any tumors. The testes, brain and liver were weighed for organ/body and organ/brain weight ratios as well as absolute organ weights.

^{*} number obtained from bibliography in WHO review on Benomyl (Nov-Dec/83) by R. Jaeger

Statistics - All data were tested with the F-test or analysis of variance, at a probability level of 5 %. Bartlett's method was used to test for heterogeneity of variances. If the variances were different, samples were tested with the Sachs' test or the Fisher-Behrens modified "t"-test.

RESULTS: There were no deaths. Clinical signs were as follows:

control No observations were noted.

0.65 mg/l The animal coats were coated with compound during the exposure period, no other observations were noted.

1.65 mg/l During the exposure period there was an aerosol cloud of high density which hindered observations. Coats were heavily coated with test aerosol during exposure. One dog had gagging, mastication and an oral mucous discharge starting 2 hr into the exposure period. All dogs were lethargic and had a white oral and nasal discharge when the exposure was terminated. One dog vomited on day 3. The remainder of the dogs appeared normal throughout the 28 day observation period.

Body weight - Although the report states there was a significant loss of weight in the high dose by 28 days, this is not evident from the data (see discussion of this review).

HDT body weight (kg)	pretest	wk 1	wk 2	wk4
all 10 dogs	10.2	10.2	10.3	
5 dogs from 28 day sac	9.6	9.7	9.7	9.5

Necropsy -There were no compound related effects observed at either the 14 or 28 day necropsy.

Organ weight - There were no organ weight changes at 14 days, however, at 28 days the HDT absolute liver weight, LDT and HDT liver to brain weight and LDT liver to body weight were significantly decreased from control values.

	control		.65	mg/l	1.65 mg/l	
	14d	28d	14d	28d	14 d	28d
brain/body wt (%)	•757	~693 [~]	•733	•757	.781	.880
liver (g)	311	370	.315	300	271	276*
liver/body wt (%)	2.86	3.22	2.74	2.60*	2.60	3.00
liver/brain (%)	378	468	375	346*	334	342*
* p <.05			<u> </u>		-	

Histology - At 14 days there was no alteration in the testes at the LDT. In the HDT animals 4/5 had reduced spermatic activity due to reduced spermatogenesis although at 28 days, no reduction was evident. There were no treatment related lesions in the other organs examined.

DISCUSSION: The high dose exposure appeared to cause mucosal irritation evidenced by a nasal and oral mucous discharge which abated after the exposure period. The only response mentioned in the report was a depressed body weight in the HDT. This however, is not evident when the initial weights of the HDT dogs is considered. Although the final (terminal sac.) 28 day weight for the HDT is 9.2 kg and the control weight is 11.5 kg (sig. at p <0.5) the pretest weights for the 5 HDT dogs was 9.6 kg and the 5 controls was 11.7 kg. The terminal weights were less for all groups than the 4 wk weights, possibly due to a prenecropsy fast (not mentioned). At the 14 day necropsy the liver (relative or absolute weight) appeared lighter at the HDT. At 28 days the liver was statistically lighter in both the LDT and HDT groups. Histologically at 14 days the spermatogenic activity was reduced. By day 28, the testes were histologically normal indicating that the depression was reversible.

CONCLUSIONS: LC50 > 1.65 mg/l (HDT) (50% WP)
No deaths occured at HDT
keduced spermatogenic activity occured at 14 days but not 28 days

TOXICITY CATEGORY: II

CORE-CLASSIFICATION: minimum

Reviewed by M.P.Copley, D.V.M. Tox. Br. 9/12/85 MRID 00066771

STUDY TYPE: 90 day feeding study - Rats

TOX. CHEM. NO .: 75A

HASKELL LAB. REPORT NUMBER: 11-67 FICHE/MASTER: 00066771

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industria!

Medicine, Wilmington, Del.

AUTHORS: H.Sherman, J.R.Barnes, W.C.Krauss, J.W.Clayton

DATE REPORT SUBMITTED: Jan. 31, 1967

TEST MATERIAL: 70 \$ wettable powder (72.2\$ tech.) Benomyl; 1-(Butylcarbamoy!)-2-benzimidazolecarbamic acid, methyl ester

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidazol-

2-yl)-methyl estar

INT-1991 NB- 5409-91 DPX-3866 N.B. 8084-166B

MATERIAL AND METHODS: Weanling albino ChR-CD rats were housed in pairs by sex, given food (with 1 \$ corn oil) and water ad libitum and observed for abnormal behavior, food consumption and weight gain for 8 days prior to test initiation. Sixteen rats per sex were placed into the following four treatment groups:

Group Treatment food + 15 CO Control (1) Low dose (V)(LDT) food + 1% CO +

100 ppm INT-1991 (0.0143% formulation) food + 1\$ CO +

500 ppm INT-1991 (0.0714≴ formulation)

food + 15 CO + High dose (VII)(HDT)

2500 ppm INT-1991 (0.357\$ formulation)

CO - corn oil

Mid dose (VI)(MDT)

Observations - Animals were observed at unspecified intervals for toxic signs, mortality and behavior throughout the study. Body weight - Animals were weighed prior to the test and twice per week thereafter.

Food consumption - Food consumption was measured prior to the test and once per week thereafter, by sex per group. Laboratory tests - They were done of 6 randomly selected rats per

sex per group at 30, 60 and 90 days. Hematology was also done prior to test initiation.

Hematology - White blood cell counts*, hemoglobin conc.*, hematocrit* and differential white blood cell count.

Urinalysis - 24 hr urine vol., conc.(m.osmols/l), protein content, sugar, ketones, pH, color, appearance and presence of occult blood. Pooled samples were used for microscopic examination.

^{*} also performed during the pretest examination

Clinical chemistries - plasma alkaline phosphatase and glutamicpyruvic transaminase activity (GPT), only tested on control and HDT.

Sacrifice - Ten male and 10 female rats* were euthanized with chloroform after 96-103 days of continuous feeding. Tissues were fixed in Bouin's solution and stained with Haskell quadrichrome. The following organs were removed for weight, fixation and staining: brain, heart, lungs, liver, spleen, kidney, testis, stomach, thymus, adrenal and pituitary. The following additional tissues were removed for fixation and staining: ovary, epididymis, Fallopian tubes, prostate, uterus, urinary bladder, duodenum, cecum, colon, skeletal muscle, peripheral nerve, bone marrow, eye, thoracic aorta, spinal cord, trachea, pancreas, thyroid, parathyriod, salivargland, and exorbital lacrimal gland.

RESULTS: Mortality - One LDT male died after 39 days, however the the registrant does not attribute the death to treatment.

Observations - There was no change in body weight, food consumption, feed efficiency, clinical signs, hematology, urinalysis, alkaline phosphatase, SGPT - except temale, organ weights (except liver - female) and histopathology from control values. The HDT female live weights were elevated over controls by 22 %:

U	Tearen	CAAT COULTEDID	Dy ZZ VI	
	Dose	liver (gm)	liver/body wt. %	
	cont.	9.50	3.40	
	LDT	9.04	2.68	
	MDT	9.40	3.14	
	HDT	_11.60 _	3.91	

The amount of test compound consumed by the rats was similar on a body weight basis for males and females. The rats consumed slightly more then one third the amount at the end of the study then at the start (mg/kg/day).

	<u>Select</u>	average d	laily in	take of	INT-1991	(mg/kg	(day	
		males				f em a	ales	
Days	cont.	LDT	MDT	HDT	cont.	LDT	MDT	HDT
U-6	0	14	73	348	0	14	66	345
41-48	0	7	36	169	0	8	39	195
83-90	<u> </u>	5	26	124	0	6	32	162

DISCUSSION: The lesions observed in the one mortality in the LDT were not attributed to the compound as no other rats had similar signs at that or higher doses. Although there were no histologic alterations in the liver, the elevated SGPT in HDT males at p < 0.001 may be biologically relevant considering the increased liver weight in the HDT females. This is difficult to assess since there were no intermediate groups tested for hepatic enzymes. Several tests required by our (Toxicology Branch) current guidelines were not performed, and no individual animal data was presented in the report. The study, however appears to be well done and the information necessary to set a NOEL is present.

*The remaining 6 rats per sex per group were used for a reproduction study reviewed separately. See Haskell report #264-68, MR #966, MRID #66773.

75

NOEL 500 ppm CONCLUSIONS:

based on increased absolute and relativer weights (female) and elevated $% \left(\frac{1}{2}\right) =\frac{1}{2}\left(\frac{1}{2}\right) +\frac{1}{2}\left(\frac{1}{2}\right) +\frac{1$ LEL 2500 ppm

SGPT levels (males).

CORE-CLASSIFICATION: minimum

Reviewed by M.P.Copley, D.V.M. Tox. Br. 9/12/85

This study has been reviewed previously by M. Quaife, 3/25/70 and L.B.Date, 10/23/68.

STUDY TYPE: 90 day feeding study - Dogs

TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 269-68

FICHE/MASTER: 00066785

MR NO :

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskall Lab. for Toxicology and Industria!

Medicine, Wilmington, Del.

AUTHORS: H.Sherman, J.R.Barnes, E.F.Stula, J.W.Clayton

DATE REPORT SUBMITTED: Nov. 20, 1968

TEST MATERIAL: 50 % wettable powder (51.5% tech.), Benomyl; !-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl estar

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzinida:

2-yi)-methyl ester

INT-1991

NB- 5409-91

DPX-3866

N.B. 8084-166B

MATERIAL AND METHODS: Beagle dogs, 7-9 months old, were given for and water ad libitum (botwoen 4 pm-7 am), observed daily for abnor behavior, signs of toxicity and weighed weekly for a month prior to test initiation. During this period, blood and urine samples were checked for the parameters listed in the lab. test section. Four males and 4 females were randomly assigned to each of the following treatment groups:

> Group Treatment Control (I) food Low dose (11)(LDT' food 100 ppm INT-1991 (.01 \$) Mid dose (III)(MDT)

food 500 ppm INT-1991 (.05 \$)

High dose (IV)(HDT) food 2500 ppm INT-1991 (.25 %)
Diets were prepared weekly and refrigerated. The HDT group was gradually given increasing amounts of INT-1991 using the following schedule: 500 ppm - 2 days; 1000 ppm - 3 days; 1500 ppm - 2 days. than 2500 ppm for the remainder of the study.

Observations - Animals were observed dally for toxic signs, morta and behavior throughout the study.

Body weight and Food consumption - Animals were weighed and food consumption measured weekly.

Laboratory tests - They were done three times during the pretest period and again at 30, 60 and 90 days.

Hematology - red blood cell count, white blood cell counts (total and differential), hemoglobin conc. and hematocrit.

Urinalysis - Urine vol. (time unspecified), osmolality, protein sugar, urobilinogen, acetone, bilirubin, pH, presence of occult blood and microscopic examination for sediment. Clinical chemistries - Glucose, urea nitrogen, cholestorol, alkaline phosphatase (AP), glutamic-pyruvic transaninase activity (GPT), total protein and albumin/globulin (A/G) rate Sacrifice - All dogs were euthanized by electrocution after 90-10' days of continuous feeding and were examined for gross and microse changes. Tissues were fixed in Bouin's solution and stained with Haskell quadrichrome. The following organs were removed for weigh fixation and staining: brain, heart, lungs, liver, splean, pancras kidney, testis, prostate, stomach, thyroid, adrenal and pitultary. The following additional tissues were removed for fixation and staining: ovary, epididymis, Fallopian tubes, uterus, urinary bladder, duodenum, cecum, colon, skeletal muscle, peripheral nurv bone marrow, eye, thoracic aorta, mammary gland, esophagus, gall bladder, spinal cord, trachea, thymus, salivary gland, and tonsil.

RESULTS: All animals survived the treatment period. There were not treatment related changes in body weight, food consumption, clinic observations, urinalysis, organ weights, gross pathology, and his pathology. Several clinical chemistry values were reported to be significantly different (p < .05) from either control or pretest values: AP for HDT males was higher than control values, GPT for HDT males was higher than pretest values, A/G for HDT males and females was lower than pretest and control values. These values, however were averages of all three time periods. All other clinic chemistry values were normal.

TEST	pretest(S.D	.) 0\$	0.01\$	0.05\$	0.251
AP(male)	2.2(0.7)	1.8*	2.0	2.4	2.5
GPT(male)	16(3.2)	23	19	18	25
A/G(male)	1.02(.19)	1.08	0.95	0.84	0.69
A/G(fmale)	1.27(.27)	1.09	0.92	1.01	0.72
"values are	avurage of	all three	time perio	ds (30, 60, 9	O days)

<u>DISCUSSION</u>: The study reports elevated AP, however when compared 'pretest values, the change was not meaningful. GPT was only elevaturing the third month while the A/G values were depressed during the entire 90 day period.

Males		pretest (range)	30 day	60 day	90 day
GPT	control	14-19	24	17	20
	HDT	12-20	20	20	29
A/G	control	.74-1.22	.98	1.10	1.16
	HDT	<u>.77-1.02</u>	.69	.69	.67

Although there appears to be a treatment related increase in cholesterol, the values are within the pretest values. Lesions reported at necropsy and histologically appears to be non-treatment related.

CONCLUSIONS: NOEL 500 ppm (.05%)
LEL 2500 ppm (.25%) consisting of increased alks phosphatase, serum glutamic-pyruvic transamainase and decreased

A/G ratio.

CORE-CLASSIFICATION: minimum

Reviewed by M.P.Copley, D.V.M. Tox. Br. 9/12/85

This study has been reviewed previously by M. Qualfe, 3/25/70

STUDY TYPE: Acute Neurotoxicity-Hens

TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: HLO 28-79

ACCESSION NO: 2419

MR NO.: 2837-001 IRDC No.: 125-028

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: International Research and Development Corporation, Mattawan, Michigan

AUTHORS: E.I.Goldenthal, R.G.Geil, D.C.Jessup, W.P.Dean, R.J.Ar

DATE REPORT SUBMITTED: Jan. 5, 1979

TEST MATERIAL: Benomyl; 1-(Butylcarbamoyl)-2-benzimidazolecarbamacid, methyl ester (% a.i. not given)

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-lH-benzimida 2-yl)-methyl ester

INT-1991 NB- 5409-91 DPX-3866 N.B. 8084-166B

Summary of study by original reviewer:

"In the first study with Benomyl, five groups of 10 White Leghorn were administered, by gavage, single doses of the following test may in 20 ml corn oil/kg respectively: 0 mg/kg (vehicle control), 750 tri-o-tolyl phosphate (TOTP, positive control), and 500 mg/kg, 250 and 5000 mg/kg benomyl (experimentals). Animals were observed for pharmacotoxic symptoms including neurotoxicity. Surviving animals sacrificed, autopsied and organs examined grossly and selected next issue from spinal cord and peripheral (sciatic) nerves examined histologically for microscopic neurotoxic effects. Weights of all were monitored during the study.

None of the vehicle controls showed any symptoms. High dose benome treated hens had one death, decreased activity and diarrhea, and some controls (altered behavior). The high dose and mid dose benomyl-treated hens (5000 mg/kg and 2500 mg/kg, respectively) shows some compound related effects on microscopic examination of spinal and peripheral nerves. Positive controls (TOTP treatment) display appropriate spectrum of nerve tissue degeneration known to be producted that chemical. However, at the conclusion of the experiment, the was declared inconclusive, due to evidence of underlying disease in hens. The disease state was determined by a group of pathologist examined the histological slide preparations of nerve tissues and identified pathological characteristics of Marek's disease."

Original reviewer, M. Sochard, Oct. 14

CORE-CLASSIFICATION: supplementary

CONCLUSION: This study can not answer the question of neurotoxic potential due to underlying disease.

Evaluation of summary by M.P.Copley, D.V. Tox. Br. 9/12/85

STUDY TYPE: Acute Neurotoxicity-Hens

TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: HLO 674-79

ACCESSION NO: 24193

IRDC No.: 125-039

GS0119-00"

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: International Research and Development Corporation, Mattawan, Michigan

AUTHORS: W.P.Dean, D.C.Jessup, R.J.Arceo, E.J.F.Spicer

DATE REPORT SUBMITTED: 10/8/79 (original), 12/6/79 (addendum)

TEST MATERIAL: Benomyl; 1-(Butylcarbamoyl)-2-benzimidazolecarbam: acid, methyl ester (99 % Tech., % a.i. not given)

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-lH-benzimida: 2-yl)-methyl ester

INT-1991 NB- 5409-91 DPX-3866 N.B. 8084-166B

MATERIAL AND METHODS: Fasted White Leghorn hens (1305-1890 gm,) months old) were given, by gavage, single doses of the following materials in 20 ml corn oil/kg:

Compound	Dose (mg/kg)	# Treated	<pre>Mortality</pre>
O(vehical cont.)	0	10	0
TOTP(pos. cont.)	1200	10	0
Benomy1	500	10	0
•	2500	10	0
•	5000	10	5(day:

TOTP - tri-o-tolyl phosphate

All hens had been vaccinated against New Castle disease, Avian Encephalomyelitis, Bronchitis and Marek's disease. They were individually housed in environmentally controlled rooms and given water and food ad libitum. After treatment they were observed twice daily for pharmacotoxic signs including neurotoxicity and weighed pretest and days 7, 14, 21. Survivors were necropsied an examined grossly. Microscopic examination was performed on selecnerve tissue from the spinal cord (3 levels) and sciatic nerve.

RESULTS AND DISCUSSION: (from the summary by M. Sochard, 10/14/81 Five of the 10 high dose benomyl treated (5000 mg/kg) hens died between 6-9 days following treatment. The deaths were considered to be the consequence of acute toxicity of benomyl. Acute neurotoxicity symptoms were seen in the high dose benomyl-treated survivors, and in TOTP-treated hens. Symptoms in high dose benow treated survivors were primarily of decreased activity. TOTP treated hens showed symptoms of delayed neurotoxicity, which benc treated hens did not. The neurotoxic behavior symptoms displayed early in the 5000 mg/kg benomy! treated hens was attributed to acute toxic effect of the chemical. Hens treated with mid and doses of benomy! (2500 mg/kg and 500 mg/kg, respectively) appear normal and behavior was normal. No treatment related gross pathological effects were seen in any hen at sacrifice and aution benomy! treated hens. Microscopic examination of spinal contant and sciatic nerves showed a spectrum of (expected) positive fine characteristic of TOTP treatment in the TOTP treated hens. Some degenerative changes were seen in negative control nerve tissue well as in some 500 mg/kg treated and 2500 mg/kg treated benom, hens — and none were seen in similar tissues from 5000 mg/kg betreated hens."

CONCLUSION: Benomy! does not appear to have delayed neurotoxi potential

NOEL for other neurotoxic signs: 2500 mg/kg

CORE-CLASSIFICATION: minimum
There were only 5 survivers in the high dose group; however, it is unlikely that it has delayed neurotoxic potential since the high and mid doses (5000 mg/kg, 2500 mg/kg) showed no histopathologic changes related to delayed neurotoxicity and this compound is not an organophosphate.

Review and Evaluation of summary by M.P.Copley, D.V.M.
Tox. Br.
9/12/85

STUDY TYPE: 21 day dermal study - rabbits TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 211-69 . FICHE/MASTER: 00097287

NO.: 1191 PATH NO. (38-69) MRID DO 097287

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industria!

Medicine, Wilmington, Del.

AUTHORS: D.B.Hood, J.R.Barnes, E.F.Stula, J.A.Zapp

DATE REPORT SUBMITTED: July 30, 1969

TEST MATERIAL: Benlate®; 1-(ButylcarbamoyI)-2-benzimidazolecarbam acid, methyl ester; powder;

SYNONYMS: a.i. Benomy!

Carbamic Acid, (1-((Butylamino)-carbonyl)-lH-benzimidaze 2-yl)-methyl ester

INT-1991 NB- 5409-91 DPX-3866 N.B. 8084-166B

MATERIAL AND METHODS: Beniate was applied topically to healthy, mature (> 3 kg) rabbits (strain unspecified). All rabbits were fitted with plastic collars and their trunks clipped prior to treatment. The study lasted 21 days (5 days treatment followed b 2 days no treatment). Test material was applied as an aqueous parents, to the clipped back (abraded daily), covered by a gauze pade taped in place (non-occlusive). After 6 hours of exposure the material was washed off and the backs were dried. The groups were as follows:

Formulation	Dose (mg/kg)*	Males	Females
	0**	5	5
	50	5	5
	250	5	5
	500	5	5
	1000	5	5
	1.000	5	5
	5000	2	2

* based on a.i.

** inert control - volume same as for the 1000 mg/kg group *** not part of the original design.

The animals were given food and water ad libitum except during the exposure period. They were observed frequently during the test period for clinical signs and weighed daily during the first week and three times a week thereafter. Prior to sacrifice, blood was taken from all animals for hemoglobin determinations, and red and white blood cell counts. The following organs were weighed: thymus, liver, kidneys, spleen, adrenal, and testes. The following

RESULTS: Body weight Only the 1000 mg/kg male group showed a decrease in weight during the first week which lasted throughout the study. The weight in the 5000 mg/kg male group was variable, although both lost weight initially, I weighed less and I more at the end of the study. There was no treatment related weight response in the females. Observations were as follows:

Dose (mg/kg)	Clinical Observations
0	mod. erythema - cracking, desquamation
50 ⁵	mild - mod. erythema
250 ^s	mild - mod. erythema
500S	mild - mod. erythema, desquamation
1000s	strong skin irrit., desquamation, dry feces
1000°	mild - mod. erythema, dry feces
5000s	mod. erythema, severe sloughing, feces dry and scant

Blood cell counts were normal. Hemoglobin concentration however, was decreased in the males at $1000^{\rm s}$ and $5000^{\rm s}$ mg/kg.

Dose	(mg/kg)	Hemoglobin	(gm/100 m1)
		13.1	
505	•	13.2	
2505	•	13.4	
500°	•	13.5	
10005		12.4	
10000		13.3	
5000S		11.9	

Organ weights - Absolute and relative testes weights were as follows:

	т	estes Wei	ghts			
Dose ⁵ (mg/k	(g) 0	50	250	500	1000	5000
individual absolute	7.44 4.90	5.11 7.50	4.20 5.90	6.11 4.00	6.71 4.30	6.25 6.50
(gm)	7.20 6.20 7.10	6.50 5.60 4.28	5.81 7.70 6.70	6.96 4.39 5.20	3.90 4.13 4.10	,
ave. abs. (gm)	6.57	5.80	6.06	5.33	4.63	6.38
ave. rel.	.185	.155	.169	.148	.140	.212

The sponsor reports a decrease in absolute testes weight only in the 1000^s mg/kg group. Aside from the one 5000^s mg/kg rabbit with with focal testicular degeneration, no treatment related <u>histopathological changes</u> were reported.

There was a treatment related decrease in male DISCUSSION: weight at the 1000s mg/kg dose level. The lack of a consistant response at the higher dose (50008 mg/kg) was probably due to the number of animals treated at that level (2), while the lack of response with the other 1000c mg/kg group may be due to the difference in formulation Skin irritation also was more severe at 1000s and 5000s mg/kg than 1000°, although the inert control group did have evidence of some irritation as well. The altered feces at 10005, c and 5000s mg/kg appeared to be treatment related although the significance of this was not addressed by the registrant. Testes weights (rel. and abs.) indicated a possible although not statistically significant, decrease at the 1000 mg/kg group with the There were only two rabbits in the 5000⁸ mg/kg group, but no decreased testicular weights were observed. Once again the difference in formulation may be responsible for the lack of testes alterations in the 1000° mg/kg. Only one rabbit with the high dose had histologic testicular alterations noted (degeneration). Although it is difficult to interpret whether this effect on the testes is treatment-related, this compound has been shown to cause inhibition of spermatogenesis in the rat by other routes of exposure (i.e. inhalation, oral).

There are additional problems with the report as presented:

1) The blood data is difficult to interpret without either standard deviations or individual animal data, 2) The Table

of organ weights for female groups 0, 50, 250 and 500 mg/kg is missing in the submitted report, however, the authors

indicated no effects on organ to body weight ratios.

CONCLUSIONS:

NOEL = 500 mg/kg

LEL = 1000 mg/kg based on decreased (not statistically testes weights (rel. and abs.).

NOEL = 1000 mg/kg (only dose tested)

The than the

appears to be more toxic at equivalant doses fromulation.

CORE-CLASSIFICATION: minimum

Review and Evaluation of summary by M.P.Copley, D.V.M. Tox. Br. 9/12/85 STUDY TYPE: Reproduction study - rats

TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 264-68(11-67) FICHE/MASTER: 00066773

MR NO : 966

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial Medicine, Wilmington, Del.

§ AUTHORS: H. Sherman

§ DATE REPORT SUBMITTED: November 18, 1968

TEST MATERIAL: Benomy!, 50 or 70% wettable powder; 1-(Buty!carbamoy!)-2-benzimidazolecarbamic acid, methyl ester; (72.2%t or 51.5-52.0%tt tech.)

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidazol-2-yl)-methyl ester

2-yl)-methyl e INT-1991

NB- 5409-91 DPX-3866

N.B. 8084-166B

" Reproduction study

Rat. 3-generation, 7-litter.

No. of Animals. 6 M and 6 F/group, F_0 parents (animals left from 90-day study); 12 M and 12 F/group, F_{1b} parents; and 20 M and 20 F/group, F_{2b} parents.

Feeding Levels.* 0, 100, 500, and 2,500 ppm.

Duration. Time to produce 3 generations, 7 litters in all.

Mortality. No effect on numbers of stillborn or on survival to 4

days or to weaning.

Body Weight. Pups from parents at 500 and 2,500 ppm weighed less, at weaning, than control or "100-ppm" pups in the F_{2b}, F_{3a}, F_{3b}, and F_{3c} litters. (See Table, below.) However, the various groups of F_{3c} pups kept on test for 9 weeks post-weaning and for a further 6 weeks on control diets had growth curves of similar slope.

Histopathology. No effect on F3b weanlings. Tissues studied were: Pituitary, thyroid, parathyroid, adrenal, skeletal muscle, sciatic nerve, brain, spinal cord, eye, exorbital lacrimal gland, mammary gland, bone marrow, spleen, thymus, lung, upper trachea, heart, stomach, duodenum, cecum, salivary gland, pancreas, liver, testis or ovary, epididymus or fallopian tube, uterus or prostate, urinary bladder, and kidney.

"No-Effect Level." Conservatively, 100 ppm; since average weanling weights in F_{2b}, F_{3a}, F_{3b}, and F_{3c} litters are low for "500-ppm" and "2,500-ppm" pups, as compared to corresponding control and "100-ppm" values.

March 25, 1970 M. Quaife, Ph.D.

§ Information obtained from the WHO bibliography (Nov.-Dec./83) on Benomyl.

t used through week 10 of the F_{1B} generation

tt used for the remainder of the reproduction study

86

PP Nos. 0F0-906 and 0G0-936

-8- -

March 25, 1970

Parameters in this reproduction study are tabulated;

	•					•			
		Average			G.1.	V.1.	L.1.	Average 'Weanling '	
	ppm	Litter Size	No. Born Alive	F.I. (%)	(%)	<u>(%)</u>	(%)	Weight ' (q)	
•	Benomy 1	3126	ATTVC						
	•			F _{la}	Litter				
	υ	11.7	11.2	100	100	94	98	48	
	100	11.2	11.2	67	100	100	100	, 54	
	500	10.2	10.0	83	100	98 ,	98	59	
	2,500	13.0	12.8	100	100	97	93	· 57	
				F _{1b}	Litter				
	0	12.5	10.8	100	100	87	98	57	
	100	13.6	13.2	83	100	97	100	58	
	500	11.6	10.6	83	100	91 1	9 3	62	
	2,500	13.2	12	100	100	91	100	54	
				F2.,	Litter		4,		
	^	10.8	10.4	83	100	9 5 '	96	51	•
	0 100	11.8	11.6	92	100	97	95	56	
	500	10.6	10.5	83	100	98	97	52	
	2,500	11.3	11.2	91	100	96	98	48	
								•	,
				F _{2b} Li	trer			ŧ.	
	0	10.8	10.0	92	91	90	99	60	
	100	13.6	13.6	92	100	100	100	59	
	500	11.1	10.6	67	100	89	97	52	
	2,500	12.9	12.6	91	90	96	100	51	
			•	F _{3a} Li	tter				
	· • o	9.5	8.9	- 85	100	93	99	56	
	100	11.3	10.7	75	93	90	98	57	
	500	9.6	9.5	70	100	98	100	52	
	2,500	11.9	11.7 ·	80	100	98	99	51	
				F _{3b} Li	tter				
	0	13.1	12.6	80	100	95	99	58	
	100	13.5	13.3	68	92	97	100	59 '.	
	500	11.1	10.7	70	100	94	99	52	
	2,500	11.9	10.4	85	100	84	98	54	
	-,500			F _{3c} Lit					
	•	11 6	10.0	65	92	87	100	60	
	0	11.6	10.5	67	100	87	100	62	
	100	11.9	8.5	. 55	100	88	93	52	
	500	9.5		75	93	79	96	51"	
	2,500	13.0	10.6	13	73	17	70	- A	

Addendum to review for clarification of material and methods by \mathbf{M}_{\bullet} . Copley.

Dietary levels of INT-1991 (using 50% WP):

 Group
 Treatment

 Control (I)
 food + 1≴ CO

 Low dose (V)(LDT)
 food + 1≴ CO +

 100 ppm INT-1991 (0.01≴ formulation)

 Mid dose (VI)(MDT)
 food + 1≴ CO +

 500 ppm INT-1991 (0.05≴ formulation)

 High dose (VII)(HDT)
 food + 1≴ CO +

 2500 ppm INT-1991 (0.25≴ formulation)

CO - corn oil

Species: ChR-CD rats.

Mating procedure: Each Fo female was exposed sequentially to 3 Fo males (from the same dietary group) for 5 days. After mating (15 days total) the females were separately housed and examined twice daily till parturition.

F_{1A} were sacrificed at weaning.

 F_{1B} - Twelve males and females from each group were mated at 3 months.

F2A were sacrificed at weaning.

F2B - Twenty males and females from each group were mated at 3 months

F3A were sacrificed at weaning.

F3B - Two of each sex from each of five litters/group were examined at necropsy. Those from the control and HDT were examined histologically.

F3C - used for reassessment of growth curve.

All litters were reduced to 10 when necessary. Parameters measured were: No. of pregnancies; no. of survivors at birth, 4, 12 and 21 days; body weight at weaning (21 days).

CONCLUSION: NOEL = 100 ppm

LEL = 500 ppm (decrease in pup weights)

CORE-CLASSIFICATION: core minimum

Original review evaluated and addendum added by M.P.Copley, D.Y.M.
Tox. Br.
9/12/85

MRID 284

STUDY TYPE: Two-year Feeding/Onco study-Rat

TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NO: 232-69(Path.No. 66-77) FICHE/MASTER: 00097284
MR NO: 966

ACCESSION NO: 05042

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial Medicine, Wilmington, Del.

AUTHORS: H.Sherman, J.R.Barnes, E.F.Stula, G.J.Stopps

DATE REPORT SUBMITTED: Aug. 15, 1969

TEST MATERIAL: Benomyl, 50 or 70% wettable powder; 1-(Butylcarbamo, 2-benzimidazolecarbamic acid, methyl ester; (72.2%) or 51.5-52.0%) tech.)

SYNONYMS: a.i. Benomy!

Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidazol

2-yl)-methyl ester

INT-1991 NB- 5409-91 DPX-3866

N.B. 8084-166B

MATERIAL AND METHODS: Male and female albino Charles River-CD strain rats were housed in pairs (by sex) and given food and water ad libitum. After a nine day observation period, healthy rats were divided into groups based on equal average weights and administered test compound in their diet by the following scheme for either 1 or 2 years (see necropsy method):

Group	No./sex/group	Dose	PPM (\$a.i.)
group 1	36	control	0 (0)
group la	36	control	0 (0)
group V	36	LDT	100 (.01\$)
group VI	36	MD T	500 (.05\$)
group VII	36	HDT	2500 (.25\$)

Observations: Animals were observed and examined regularly (internot specified) for behavioral and toxicological abnormalities.

Food Consumption and Weight: Animals were weighed once/week for 1. months than twice/month for the remainder of the study. Food consumption was monitored for the same intervals by sex and group. Laboratory Studies: Hematology - Six randomly selected rats/sex/group were tested at pretest, 1, 3, 6, 9, 12, 18 and 24 months for hematocrit (HCT), hemoglobin (Hg), RBC count, WBC count and WBC differential count*. Urinalysis (UA) - Urine was collected over a 24 hour period from the animals used for hematology (no pretest UA) and examined with respect to the following: protein, sugar, blood, pH, ketone bodies, volume, solute concentration (mosmoles/1) color, appearance and microscopic abnormalities. Clinical Chemistr Ten randomly picked male and females rats in the 0, 500 and 2500 p; groups were tested after 1, 3, 6, 9, 12, 18 and 24 months for plasm alkaline phosphatase. Serum glutamic-pyruvic transaminase (GPT)

t used for the first 8 weeks of the srudy

tt used for the remainder of the feeding study

* only on controls, .05% and .25% animals

was tested only in the control and HDT groups unless elevated levels were detected.

Necropsy: There was an interm i year sacrifice with gross and microscopic pathologic examination reducing each sex/group to 30 animals. After 2 years, the surviving rats were also sacrificed. Tissues were fixed in Bouin's solution, stained with Haskell quadrichrome and examined microscopically. All the listed tissues from the control and HDT (12 and 24 months) rats were examined initially, while only liver, kidney and testes were evaluated in the LDT and MDT at 24 months. Subsequently however, all 24 month tissues were examined histologically and reported in Supplemental Pathology Report No. 66-77.

tbrain
theart
tkidney
tadrenal
ovaries
tstomach
eye
skeletal muscle
urinary bladder
salivary gland
exorbital lacrimal gland

tliver
pituitary
epididymis
lymph node
peripheral nerve
fallopian tube
tspleen
thyroid, parathyroid
prostate
ttestes
uterus

thoracic aorta
thymus
bone marrow smear
lumber spinal cor
trachea
tlung
pancreas
duodenum
cecum
colon

torgan weights, all groups, 12 and 24 months

RESULTS: taken from original review.of.March 25, 1970 by M.Quaife.

Chronic toxicity studies

Rat, 2-year feeding.

No. of Animals. 36 M and 36 F/group.

Feeding Levels. 0, 0 (second control group), 100, 500, and 2,500 ppm.

Duration. 2 years. Mortality. No effect.

Body Weight. No significant effect. (None on food consumption or food efficiency, either.)

General Behavior. No effect. No clinical signs of toxicity attributed - to effect of benomyl.

Organ Weight. No effect on weights (either absolute or relative to body weight) of brain, heart, lungs, liver, spleen, kidneys, testes, stomach, adrenals, and pituitary.

Clinical Laboratory Tests. No effect on alkaline phosphatase or serum · ; glutamic-pyruvic transaminase determined in rats of both control ·

^{*} Test substance was 70% or 50% wettable powder formulated as given, above, for either INT-1991-30 or INT-1991. Dietary levels based on active ingredient.

groups and those at 500 or 2,500 ppm at 0, 1, 3, 6, 9, 12, 18, and 24 months on test. No effect on hematologic values (same ones as determined in rat 90-day study at time intervals given in preceding sentence). No effect on results of urinalysis (also done at same time intervals): Volume; solute concentration; levels of sugar, protein, and ketone bodies; color; pH; presence of occult blood; and microscopic appearance of urinary sediment.

Histopathology. Tissues examined histologically, in addition to those listed under "organ weight," above, ara: 'Ovary, epididymus, fallop tuhe, prostate, uterus, urinary bladder, duodenum, cecum, colon, skeletal muscle, peripheral nerve, bone marrow, eye, thoracic aorta, lumber spinal cord, traches, thymus, pancreas, thyroid, parathyroid salivary gland, lymph node, and exorbital lacrimal gland. These tissues from control groups and from 2,500-ppm group examined at both 1 and 2 years. At 2 years, liver, kidney, and testis of 100and 500-ppm groups also studied. No significant findings believed related to intake of test compound, benomyl, were made. Validity of this opinion is verified by K. Davis, DVM, Pathologist (told to . M. Quaife on January 20, 1970). We note that only certain tissues of animals in one of the control groups were studied. In male rats of the other control group (IA), there was a very high incidence of pituitary tumors and chronic nephritis (> 85% each); such incidence of pituitary tumors is not matched in the 2,500-ppm male rats. Livchanges were of frequent occurrence but about equally spread between control and test groups. Likewise, for testicular degeneration in male rats.

Neoplasms. No effect.

"No-Effect Level," 2,500 ppm. "

Results in the supplementary pathology report with histology for all rats on test indicated no increased incidence of either neople or non-neoplastic lesions.

DISCUSSION: AP and GPT were the only clinical chemistries performed on the rats making it difficult to confirm the NOEL for toxicity. There was however, no reason to expect chemistry changes since there were no compound related organ weights or histopathologic changes in any of the groups tested at either sacrifice time.

CONCLUSIONS:

NOEL chronic feeding > 2500 ppm NOEL oncogenicity > 2500 ppm

CORE-CLASSIFICATION:

Chronic feeding - minimum

Oncogenicity - supplementary since no MTD was established

Original review evaluated and addendum added by M.P.Copley, D.V.M. Tox. Br. 9/12/85

STUDY TYPE: Primary Eye Irritation - Rabbit

TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NO: 497-80

MR NO.: 0581-867

FICHE/MASTER: 00064820 ACCESSION NO.: 243043-

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial Medicine, Wilmington, Del.

AUTHORS: O.L.Dashiel, P.Dashley, G.L.Kennedy

DATE REPORT SUBMITTED: June 13, 1980

TEST MATERIAL: Benlate Dry Flowable (75% a.i.); 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester

SYNONYMS: a.i. Benomyl

Carbamic Acid, (1-((Butylamino)-carbonyl)-lH-benzimidazol-

2-yl)-methyl ester

INT-1991 DPX-3866

N.B. 8084-166B

Original review

Eye Irritation in Rabbits; Haskell Report #497-80; June 13, 1980; Acc. No. 243043

<u>Procedure</u>: "Benlate OF Fungicide" was applied into one eye of each of 9 albino rabbits. In each case, 0.1 ml of the test substance was applied. Three of the animals' eyes were irrigated with tap water for one minute, 20 seconds post-treatment.

Results: In the non-irrigated eyes at 24 hours, corneal opacity observed 10.2/6=5, 1/6=10, 1/6=20, 2/6=40; iris irritation in 3/6=5; conjunctival redness in 5/6=1; chemosis in 5/6=1 and discharge in 3/6=1. By day 8.7 he only irritation was slight corneal opacity (1/6=5, 1/6=10). All non-irrigated eyes were clear by day 11. Three animals showed injury when viewed with biomicroscope at 8 days. For the irrigated eyes at 24 hours, corneal opacity exhibited in 1/3=5, 2/3=10; conjunctival redness in 2/3=1. All irritation cleared by day 8.

Study Classification: Core Guideline Data.

Toxicity Category: II-WARNING. In this case 2/6 non-irrigated eyes exhibited corneal opacity, 3/6 animals showed corneal injury when viewed with biomicroscope all at day 8.

Original review by Sherell A. Sterling FHB/TSS 11/14/80

(addendum on next page)

Addendum by M.P.Copley

MATERIAL AND METHOUS:

Test material (0.1 ml, 51.4 gm) was instilled in the right eyes. Eyes were examined and scored according to the method of Draize σ days 1, 2, 3, 4, 8, and 11.

CONCLUSIONS: Unwashed eyes: Benlate produced corneal opacities which were reversible by 11 days. Mild iritis and conjunctivities were present for only 3 days. Washed eyes: Washing after 20 see was effective in decreasing corneal lesions and keeping conjunction a minimum.

Eye irritation score:

	1d	2 d	_3d	_ 4 d	8 d	_11d	
unwashed	27	17	9	7	3	0	_n≖€
washed	10	9	7	2	0	0	n = 3

Original review evaluated and addendum added by M.P.Copley, D.V. Tox. Br. 9/12/85

STUDY TYPE: Primary Eye Irritation - Rabbit TOX. CHEM. NO.:

HASKELL LAB. REPORT NO: 179-81

MR NO.: 0581-935

FICHE/MASTER: 00 ACCESSION NO.: 2

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industri

Medicine, Wilmington, Del.

AUTHORS: L.S.Silber, O.L.Dashiel, G.L.Kennedy

DATE REPORT SUBMITTED: April 6, 1981

TEST MATERIAL: Beniate Dry Flowable (75% a.i.); 1-(Butylcarbamo 2-benzimidazolecarbamic acid, methy' ester

SYNONYMS: a.i. Benomy I

Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimid

2-yl)-methyl ester

INT-1991 DPX-3866

N.B. 0759-85-2

MATERIAL AND METHODS: Test material (0.1 ml, 46.2 mg) was instin the right eye of 9 male albino rabbits (strain unspecified). Twenty seconds later 3 eyes were washed with tap water for 1 mi The left eyes were used as untreated controls. Eyes were examiscored according to the method of Draize on days 1, 2, 3, 4 and

RESULTS: Unwashed eyes: Corneal opacities persisted 24 hr in ! animal, 2d in 2 rabbits and 3d in 3 rabbits. All unwashed eyes were normal by 4 days. Minimal Iritis occurred in 5 animals and disappeared after 4 days. Conjunctival Irritation, characteris by minimal redness, chemosis and a bloody discharge (day 1 only was present in all 6 eyes for no more than 2d. Individual animal scores ranged from 14-55 on day 1 and 0-29 on day 2. Biomicros corneal effects were moderate to slight on day 1 and disappeared ay 4. Washed eyes: Washing after treatment decreased lesions minimal corneal opacities lasting only 3d in 2 animals. The thrabbit had lesions lasting till day 4, most severe on day 3. Conjunctival redness occured in 1 rabbit lasting 2 days. Biomicorneal effects were slight in all 3 rabbits lasting through day

CONCLUSIONS: Unwashed eyes: Beniate produced slight to mild coopacities which were reversible by 7 days. Minimal iritis and conjunctivitis were present for 4 days. Washed eyes: Washing after 20 seconds was effective in decreasing corneal lesions an keeping conjunctivitis to a minimum in 2 out of 3 animals. One had lesions as severe as these without washing.

Eye irritation score:

	<u>1d</u>	2d _	3 d	4 d	7 d	
unwashed	28.1	14.2	4.2	7.3	0	_n=6
washed	4.7	4.7	13.3	3.3	0	n=3

Toxicity Category: 111

CLASSIFICATION OF STUDY: core-minimum

Reviewed by M.P.Copley, D. Tox. Br. 9/12/85

STUDY TYPE: Skin Irritation - Rabbit

TOX. CHEM. NO .:

HASKELL LAB. REPORT NO: 367-80

MR NO .: 0581-867

FICHE/MASTER: 0006 ACCESSION NO .: 242

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industria

Medicine, Wilmington, Del.

AUTHORS: O.L.Dashiel, L.S.Silber

DATE REPORT SUBMITTED: May 12, 1980

TEST MATERIAL: Benlate Dry Flowable (75% a.i.); 1-(Butylcarbamoy

2-benzimidazolecarbamic acid, methyl ester

SYNONYMS: a.i. Benomy!

Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimida.

2-yl)-methyl ester

INT-1991 DPX-3866

N.B. 8084-166B

Original review

Skin Irritation Test on Rabbits; Haskell Report #367-80; May 12, 1980; Acc. No. 243043

Procedure: 6 New Zealand white rabbits were exposed to the "Benlate DF Fungicide" at 4 sites on each rabbit (2 abraded, 2 intact). The test substance was applied as a paste at 0.5g per site under occlusive wrap fc 24 hours. Animals were observed at 24, 72 hours, 6 days.

Results: At 24 hours intact sites showed erythema in 6/12=1, 2/12=2; no edema. Abraded sites at 24 hours exhibited erythema in 9/12=1, 2/12=2; edema in 6/12=1, 1/12=2. By 72 hours, only 1/12 showed very slight erythema at abraded sites; no irritation at intact sites. All scores wer 0 by day 6.

Study Classification: Core Guideline Data.

Toxicity Category: IV - CAUTION "

Original review by Sherell A. Sterling; FHB/TSS; 11/14/80

Addendum

CONCLUSION: Benomy! (75% a.i.) is a slight to mild irritant

PIS: 0.67 (range 0.25 - 1.0) for day 1

Original review evaluated and addendum added by M.P.Copley, D.V.M.

Tox. Br.

×9/12/85

96

STUDY TYPE: Skin Irritation and

sensitization - Guinea Pigs

TOX. CHEM. NO.: 7

HASKELL LAB. REPORT NO: 84-69

MR NO.: 0581

FICHE/MASTER: 0009 ACCESSION NO.: 504.

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial

Medicine, Wilmington, Del.

AUTHORS: C.W.Colburn, J.A.Zapp

DATE REPORT SUBMITTED: April 18, 1969

TEST MATERIAL: Benomyl (Technical); 1-(Butylcarbamoyl)-

2-benzimidazolecarbamic acid, methyl ester

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidaz

2-yl)-methyl ester

INT-1991-202 DPX-3866 N.B. 6275-175

MATERIAL AND METHODS: Skin irritation: Ten male albino guinea piwere treated with .05 ml each of a 10%, 25% and 40% paste of Benoin dimethylphthalate (DMP). The material was rubbed into shaved intact skin and scored 24 hr later using the following system:

0	- negative	
+	- mild erythema	
++	- moderate erythema	
+++	- strong erythema	
++++	- erythema with edema	
Sensitized	S) - presence of reaction beyond site of applica-	t

Sensitization: Induction - The same ten animals were used as foll 5 received 8 applications of the 40% and 1 of 26.6% paste rubbed on abraded skin over a 3 week period. The remaining 5 animals received 4 intradermal injections of a 1% solution in DMP (.1 ml) Challenge - The animals received the 1st challenge after a 2 week rest period. Twenty-five and 10% pastes were applied to both intact and abraded skin on the induced animals and scored 24 hr later. Challenge 2 was given 5 days later. Ten controls without prior induction also received both challenges.

<u>RESULTS</u>: The following are the results for the irritation and sensitization tests:

dose	irri	tat	ion*		cha	lleng	e 1**			С	halle	nge_2**	-
level	0	+	++ -	+	++	+++	++++	S	0	+	++	+++ ++	++
10%	2	7	1	7/5	2/4	1/	/1	6	5/3	4/5	/1	1/1	
25%	1	8	1	3/1	5/5	/1	2/3	1	1/1	6/6	2/2	1/	1
40%	7	3_											

^{*} number of animals affected

^{**} number of intact sites/number of abraded sites

-41-

004679

Control animals had:

dose	intact		abra	ded
level	0	+	0	+
10%	9	1	6	4
25%	6	4	8	2

DISCUSSION: This compound is a mild to moderate irritant in guin pigs. Although it is a moderate sensitizer the sensitization decreases rapidly. Individual animal data was not presented by tregistrant.

CONCLUSIONS: Moderate sensitizer

Mild irritant

CORE-CLASSIFICATION: minimum

Reviewed by M.P.Coplay, D.V. Tox. Br. 9/12/85

STUDY TYPE: Mutagenicity Evaluation in

TOX. CHEM. NO.: 7

Salmonella Typhimurium

HASKELL LAB. REPORT NO: 560-80

FICHE/MASTER: GS01:

MR NO .: 0581-881

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industria:

Medicine, Newark, Del.

<u>AUTHORS</u>: A.L.Horst , D.F.Krahn

DATE REPORT SUBMITTED: Aug. 22, 1980

TEST MATERIAL: Benomy! (99.6% a.i.); 1-(Butylcarbamoy!)-2-

benzimidazolecarbamic acid, methyl ester

SYNONYMS: a.i. Benomy!

Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimida:

2-yl)-methyl ester

INT-1991 DPX-3866

Original review

"Horst and Krahn (1980) evaluated the mutagenic potential of technical grade benomy! (Polish; Cieck, 97.6% pure) in S. Typhimur Plates were treated with dosages ranging from 100 to 10,000 ug in the presence or absence of liver microsomal activation. The 5,00 and 10,000 ug doses with activation increased the number of rever plate over that of control plates by 2.6 and 7.6 times in strain TA 1537, respectively. The same two doses caused respective incr of 2 and 10 times (above)* controls in strain TA 98."

Original review by Roger Gardner, TB, 7/6/82

Addendum

MATERIAL AND METHODS: Strains TA 1535, TA 1537, TA 98 and TA 100 were tested in 2 independent trials each with duplicate plates. was no cytotoxicity observed in TA 1535 at concentrations up to 10,000 ug/plate.

CONCLUSION: Test material is mutagenic in S. typhimurium with activation.

CLASSIFICATION: Acceptable

Original review evaluated and addendum added by M.P.Copley, D.V.M. Tox. Br. x9/12/85

STUDY TYPE: Chinese Hamster Ovary Cell Assay TOX. CHEM. NO.: 75

(HGPRT)

HASKELL LAB. REPORT NO: 438-80 FICHE/MASTER: 0003

MR NO .: 581-852

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industria!

Medicine, Newark, Del.

AUTHORS: K.Fitzpatrick, D.F.Krahn

DATE REPORT SUBMITTED: May 16, 1980

TEST MATERIAL: Benomy! (99.9-100% a.i.); 1-(Butylcarbamoy!)-

2-benzimidazolecarbamic acid, methyl ester

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidazo

2-yl)-methyl ester

INT-1991 DPX-3866

Original review

"A report on a mutagenicity assay with a Chinese hamster ovar cell line which can demonstrate mutations at the gene locus coding for hypoxanthine-guanine phosphoribosyl transferase (HGPRT) was submitted (Fitzpatrick and Krahn, 1980). This study included benzo(a)pyrene and ethyl methane sulfonate as positive controls an a vehicle control (DMSO) were used, and benomyl was added to test cultures with or without metabolic activation by rat liver microso enzymes (S-9). Resistance of cells to 6-thioguanine was used as the Indicator of mutagenic effects.

The authors reported a dose-related cytotoxic response which was more evident in cultures exposed to the chemical without activation. No statistically significant differences in mutation frequency were noted in cultures treated with activated or nonactivated benomy!. Concentrations ranged from 17 to 172 uM, an no statistically significant trends were noted. Positive controls demonstrated that the test system was sensitive, and cell survival was greater than 10% at most concentrations used. The authors concluded that benomy! was not mutagenic under these test conditio (Fitzpatrick and Krahn, 1980)."

Original review by Roger Gardner; TB; 7/6/82

Addendum

<u>MATERIALS AND METHODS</u>: Benomy! was tested in 2-5 independent trials each with duplicate plates.

CONCLUSION: Test material is not mutagenic in this test system

CLASSIFICATION: Acceptable

Original review evaluated and addendum added by M.P.Copley, D.V.M. Tox. Br. 9/12/85

STUDY TYPE: Mutagenesis - L5178Y TK+/-

TCX. CHEM. NO.: 7

LAB. PROJECT NO: LSU-7558

FICHE/MASTER:

SPONSOR: Environmental Protection Agency

STUDIES PERFORMED AT: SRI International, Menio Park, California

AUTHORS: M.M.Jotz, D.D.Rundle, A.D.Mitchell

DATE REPORT SUBMITTED: Dec. 1980

TEST MATERIAL: Benomyl (99% a.i.); 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidazo

2-yl)-methyl ester

INT-1991 DPX-3866

Original review

"The ... study (Jotz, 1980) evaluated the ability of benomy! with and without metabolic activation (liver S-9 mix) to induce forward mutations at the thymidine kinase (TK) locus in mouse L5178Y lymphoma cells, ethylmethane sulfonate (EMS) and 3-methylcholanthrene were used as positive controls.

The authors concluded that benomyl is mutagenic in this test system since the mutation frequency was increased in a dose-relate manner, and some doses which had cytotoxicity increased the mutation frequency by more than twice that of the vehicle control (DMSO). The table summarizes the results for the controls and highest dose causing less than 90% cell death. The results indicated that metabolic activation enhanced benomyl's mutagenic activity."

Original review by Roger Gardner; TB; 7/6/82

Addendum

MATERIALS AND METHODS: Concentrations without activation were from 2-25 ug/ml and with activation were 5-50 ug/ml, with higher doses causing less than 10 % growth. The range was determined based both on relative total growth and precipitation. There was one trial with duplicate samples.

CONCLUSION: Increased mutation frequencies were observed at 50 ug without activation and at 12 and 25 ug/ml with activation. The tematerial is a weak mutagen in this system.

CLASSIFICATION: Acceptable

Original review evaluated and addendum added by M.P.Copley, D.V.M. Tox. Br. 9/12/85 "The Effects of Benomyl on the Frequency of Forward Mutations at the TK Locus in L5178Y Mouse Lymphoma Cells (Jotz et al., 1980) 1/

Test Group	Relative Total Growth Percent	Mutation Frequency (x 10 ⁻⁶),2/
Without Activati	on	
Vehicle Control	107.2	38
(DMSO 1%)	93.1	37
Positive Control		
(EMS 1500 .	35.2	655
ug/ml)	48.2	449
Benomyl	18.8	122
$(50 \text{ ug/ml})^{3/}$	23.6	90
	With Ac	tivation
Solvent Control	103.4	66
(1% DMSO)	96.5	91
3-methylcholanthrene	50.8	316
(5 ug/ml)	58.3	342
Benomyl	9.1	531
(25 ug/ml) <u>4</u> /	10.5	583

^{1/}Replicate results are reported.

 $[\]frac{2}{\text{Determined}}$ by dividing the number of mutant colonies seen by the umber of potentially viable colonies per 3 x 10^6 cells plated.

^{3/}Higher doses caused relative total growth to be less than 10%, and lower doses did not consistently cause mutation frequencies of twice the control rate.

 $[\]frac{4}{\text{The highest dose tested.}}$ Doses as low as 12 ug/ml doubled the mutation frequency when compared with controls."

STUDY TYPE: Micronucleus test

TOX. CHEM. NO.: 75/

LAB. PROJECT NO: LSU-7558-19

FICHE/MASTER:

SPONSOR: Environmental Protection Agency

STUDIES PERFORMED AT: SRI International, Menio Park, California

AUTHORS: B. Kirkhart

DATE REPORT SUBMITTED: Feb. 12, 1980

TEST MATERIAL: Benomy! (% a.i. not given); 1-(Butylcarbamoy!)-

2-benzimidazolecarbamic acid, methyl ester

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidazo:

2-yl)-methyl ester

INT-1991 DPX-3866

Original review

"In the ... study, groups of 24 male mice were given daily doses by gavage of 250, 500, or 1,000 mg benomy! per kg body weight on two consecutive days. A vehicle control (DMSO) group was also included. The authors stated that the highest dose presented a solubility problem which was corrected before the second dosage was administered, but they were uncertain about the amount given the first time. Eight animals from each group were sacrificed 24, 48, or 72 hours after the second dose was administered. Bone marrow from the femur of each animal was taken for examination. For each animal, 500 polychromatic erythrocytes (PCE) were examined for micronuclei, and the number of mature erythrocytes was counted until 200 PCE's were found.

The authors stated that a compound is considered to be positive in this assay if at least two dose-time groups had a statistically significant increase over controls in the number of cells with micronuclei per 500 PCE's. Before the study is considered negative at least 4,000 PCE's per dose-time group must be examined at 24 to 96 hours after the first dose is given; the highest dose is a maximum tolerated dose; the average PCE to erythrocyte ratio is greater that 0.15 for each group and all groups do not have a statistically significant increase in the number of cells with micronuclei per 500 PCE's.

According to the authors, the statistical procedure used shown that four groups had significantly increased numbers of cells with micronuclei. These groups included the low and mid dose groups at 48 hours (15/3500 and 16/4000, respectively as compared to 5/3000 cells from vehicle controls) and in the high dose group at 48 and 72 hours after treatment (20/3500 and 17/3500, respectively; respective control values are 5/3000 and 6/3500)."

Original review by Roger Gardner; TB; 7/6/82

Addendum

CONCLUSION: Test material is mutagenic in this system

CLASSIFICATION: Acceptable

Original review evaluated and addendum added by M.P.Copley, D.V.M.
Tox. Br.
9/12/85

.....

STUDY TYPE: Sister Chromatid Exchange (CHO)

TOX. CHEM. NO.: 7'

LAB. PROJECT NO: LSU-7558

FICHE/MASTER:

SPONSOR: Environmental Protection Agency

STUDIES PERFORMED AT: SRI International, Menio Park, California

AUTHORS: E.L.Evans, A.D.Mitchell

DATE REPORT SUBMITTED: Aug. 1980

TEST MATERIAL: Benomy! (99 % a.i.); 1-(Butylcarbamoy!)-

2-benzimidazolecarbamic acid, methyl ester

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidaze

2-y1)-methyl ester

INT-1991 DPX-3866

Original review

"The ... study (Evans and Mitchell, 1980) was conducted with Chinese hamster ovary cells in cultures containing varying concentrations of benomyl without metabolic activation (0, 0.625, 1.25, 2.5, 5 or 10 ug/ml) or metabolically activated benomyl (0, 0.375, 18.75, 37.5, 75 or 150 ug/ml)... These concentrations were selected so that treated cells could undergo at least two division within 24 hours. Ethyl methane-sulfonate (EMS) and dimethylnitros (DMN) were used as positive controls and a vehicle control (0.95 a ethanol in culture medium) was included. Two samples of 25 cells each were scored for number of sister chromatid exchanges and number of chromosomes. A total of 50 cells were scored for each group.

The 5 and 10 ug/ml dosages did not allow sufficient numbers of second division metaphases to occur for an evaluation. The author stated that there was a scoring discrepancy between the two cytogeneticists' observations. One found no effects, while the second noted an increase in sister chromatid exchanges (SCE) which peaked at the 1.25 ug/ml concentration. A third cytogeneticist's observations were reported to show a plateau in the increased number of SCE's at the three concentrations. Analysis of variance applicational three sets of observations showed a statistically signification activation (variance between groups was significantly greater than that within groups). Similar results were obtained when three sets of observations were analyzed for metabolically activated benomy!

The number of SCE's (per chromosome) in the EMS group was three to four-times that of the unactivated negative controls, while the three unactivated benomyl groups had one-third more SCE' (per chromosome) than controls. In the experiments with activated benomyl, the DMN positive controls had approximately twice the number of SCE's found in negative controls. The benomy groups had increased numbers of SCE's (by approximately 25 to 100%)

above controls). The number of SCE's per cell was increased by or sixth to one-half above that for negative controls for unactivated benomyl. The activated fungicide increased that number by approximately 50 to 100% over that seen in controls.

The authors concluded that results of this study were weakly positive (Evans and Mitchell, 1980)."

Original review by Roger Gardner; TB; 7/6/82

Addendum

CONCLUSION: Test mater al is weakly mutagenic in this system

CLASSIFICATION: Acceptable

Original review evaluated and addendum added by M.P.Copley, D.V.M.
Tox. Br.
9/12/85

STUDY TYPE: DNA Repair (I mouse hepatocytes) TOX. CHEM. No.:

HASKELL LAB. REPORT NO: 741-81

FICHE/MASTER:

MR NO .: 4065-001

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industria

Medicine, Wilmington, Del.

AUTHORS: C. Tong

DATE REPORT SUBMITTED: Oct. 20, 1981

TEST MATERIAL: Benomy! (\$ a.i. not given); 1-(Butylcarbamoy!)-

2-benzimidazolecarbamic acid, methyl ester

<u>SYNONYMS</u>: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimida

2-yl)-methyl ester

INT-1991

DPX-3866

N.B. 8084-166B

Original review

"The DNA repair assay in mouse hepatocyté primary cultures (HPC) was submitted (Tong, 1981). Benomyl ... was evaluated at with dimethylnitrosamine, dimethylformamide, fluorene and 2-amin fluorene which were used as positive controls. The liver was removed and primary cultures were started with hepatocytes from B6C3F1 mice benomy! and tritiated thymidine (19 uCi) were added to the culture medium. After 18 to 20 hours of incubating the treated cultures, they were fixed and examined microscopical for morphological changes and absence of s-phase nuclei indicati of cytotoxicity. Autoradiographic techniques were used to deter the number of nuclear grains induced by test chemicals. Backgro counts were obtained by evaluating three nuclear-sized areas in cytoplasm; these values were averaged and subtracted from the nu counted in the nucleus to obtain a net value for each nucleus. chemical is considered capable of inducing DNA repair when a net count of 5 grains or more is observed consistently in each of th nuclei examined in each of 3 replicate experiments.

Benomyl did not induce DNA repair in ... mouse hepatocytes (Tong, 1981) The dimethylnitrosamine and 2-amino fluorene increased the number of nuclear grains from 7 to 15 times the 1σ set as the criterion for a positive response (5/nucleus; Tong, i

Original review by Roger Gardner; TB; 7/6/82

Addendum

MATERIAL AND METHODS: Five log doses were tested in triplicate independent tests (.5, .05, .005, .0005, .00005 mg/ml). Only th non-toxic levels were counted.

RESULTS: Test 1: .5 and .05 mg/ml were toxic

Test 2: .5 and .05 mg/ml were toxic

CONCLUSION: Test material is not mutagenic in this system

CLASSIFICATION: Acceptable

Original review evaluated and addendum added by M.P.Copley, D.V.M.
Tox. Br.
9/12/85

STUDY TYPE: DNA Repair (1° rat hepatocytes)

TOX. CHEM. NO.:

HASKELL LAB. REPORT NO: 741-82

FICHE/MASTER: GS0119-006

MR NO .: 4065-001

950115-00

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industri

Medicine, Wilmington, Del.

AUTHORS: C. Tong

DATE REPORT SUBMITTED: Oct. 20, 1981

TEST MATERIAL: Benomy! (% a.i. not given); 1-(Butylcarbamoy!)-

2-benzimidazolecarbanic acid, methyl ester

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimid-

2-y1)-methyl ester

INT-1991 DPX-3866

N.B. 8084-166B

Original review

"The DNA repair assay in rat hepatocyte primary cultures was submitted (Tong, 1981). Benomy! ... was evaluated a with dimethylnitrosamine, dimethylformamide, fluorene and 2-ami fluorene which were used as positive controls. The liver was removed and primary cultures were started with hepatocytes from F344 rats benomy! and tritiated thymidine (19 uCi) were added t the culture medium. After 18 to 20 hours of incubating the trecultures, they are fixed and examined microscopically for morphological changes and absence of s-phase nuclei indicative of cytotoxicity. Autoradiographic techniques are used to deterthe number of nuclear grains induced by test chemicals. Backgr counts were obtained by evaluating three nuclear-sized areas in cytoplasm; these values were averaged and subtracted from the n counted in the nucleus to obtain a net value for each nucleus. chemical is considered capable of inducing DNA repair when a necount of 5 grains or more is observed consistently in each of t nuclei examined in each of 3 replicate slides.

Benomyl did not induce DNA repair in ... rat hepatocytes (Tong, 1981) The dimethylnitrosamine and 2-amino fluorene increased the number of nuclear grains from 7 to 15 times the liset as the criterion for a positive response (5/nucleus; Tong,

riginal review by Roger Gardner; TB; 7/6/82

ddendum

MATERIAL AND METHODS: Five log doses were tested in triplicate independent tests (.5, .05, .005, .0005, .00005 mg/ml). Only transfer levels were counted.

RESULTS: Test 1: .5 mg/ml was toxic Test 2: .5 and .05 mg/ml were toxic

CONCLUSION: Test material is not mutagenic in this system

CLASSIFICATION: Acceptable

Original review evaluated and addendum added by M.P.Copley, D.V.M. Tox. Br. 9/12/85

MA18674

004679

STUDY TYPE: Teratology - Rats (microphthalmia) TOX. CHEM. NO.: 75

HASKELL LAB. REPORT NUMBER: 587-82

FICHE/MASTER: 00115674 ACCESSION: 248563 249749

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial

Medicine, Wilmington, Del.

AUTHOR: R.E.Staples

DATE REPORT SUBMITTED: 1982

TEST MATERIAL: Benomyl; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic

acid, methyl ester

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-lH-benzimidazol-

2-yl)-methyl ester; (99.1% purity)

INT-1991-474 DPX-3866 N.B. 5103-109

Reviewed by Roger Gardner, June 30, 1983

" Materials and Methods:

Test substance: Benomyl (99.1% purity, contaminants were not identified) was used. The sample was numbered INT-1991-474, N.B. 5103-109, Lot F00117E

Test Species: Pregnant Crl: CD® (SD) BR rats were used. Day 1 of gestation was the day sperm were detected in vaginal smears.

Experimental Procedure:

Benomyl was suspended in stripped corn oil and administered by gavage at dosages of 0, 3, 6.25, 10, 20, 30, or 62.5 mg/kg. The dosages were administered in 1 ml of vehicle daily from day 7 through day 16 of gestation. There were 46, 47, 47, 48, 47, 47, or 19 animals in the 0, 3, 6.25, 10, 20, 30, or 62.5 mg/kg/day dose groups, respectively.

On day 21 of gestation dams were sacrificed and examined for gross pathological signs. Ammonium sulfide solution was used to determine the incidence of pregnancy in uteri of apparently non-pregnant dams.

Maternal body weights were obtained on day 5 of gestation for the purpose of dosage preparation.

At sacrifice the numbers of implantation sites, resorptions, live fetuses and dead fetuses were determined. Fetuses were individually weighted and mean live fetus weights per litter were calculated.

Fetal examinations were limited to the determination of the incidence of external hydrocephaly and microphthalmia. Eye diameters were measured in cases of suspected asymmetrical or small eyes. One measurement was made from the pinna through the center of the eye, and the other was made through the center and perplendicular to the first. The criteria for identification of microphthalmia considered the smaller of the two measurements. If both measurements were at least 0.4 mm less than those in the alternate eye, the smaller eye was classified as microphthalmic. Both eyes of a fetus were classified microphthalmic if the measurement was less than 1.8mm (the smallest diameter found in the control group). A transverse section through the center of both eyes was made freehand, and the eyes were examined for microphthalmia. All measurements and examination were made under magnification (10X).

A transverse section was made throught the widest portion of the head which was then examined for signs of internal hydrocephaly.

The author noted that the litter was considered the experimental unit for statistical analyses. The analyses included the Fisher's exact test for incidence of maternal and fetal mortality and occurrence of fetal effects, the Mann-Whitney U test for significant differences in maternal body weights, one-way analysis of variance and Dunnett's tests for maternal body weights after censoring those animals without live fetuses, dying before scheduled sacrifice, or those bred on the wrong date, and Jonckheere's test for significance of dose-response relationships.

Reported Results:

The author noted that no statistically significant differences between group mean maternal body weights were found.

One dam died on day 11 of gestation because of dosing error (30 mg/kg/day group). Other dams were excluded from the study because of errors in breeding date estimation (detected on the basis of unusually light or heavy litter weights). There were 4, 2, 3, 2, 3, 3, or 1 eliminated from the 0, 3, 6.25, 10, 20, 30 or 125 mg/kg/day groups, respectively.

Pregnancy rate varied from 84.2% (16/19) in the highest dose group to 95.7% (44/46) in the control group. No statistically significant differences were noted by the authors. Only one fetus was found dead (10 mg/kg/day). The highest dose group was reported to contain 1 fetus with microphthalmia and 1 fetus with hydrocephaly in separate litters). The number of litters containing fetuses with hematomas was comparable in the control and treated groups with the exception of the highest dose. In that dose group 11 of 16 litters contained an average of 11 (+6.0) % fetuses with hematomas (1 to 2 per litter) while 15 of 43 control group litters contained fetuses with hematomas (1 to 2 per litter). Mean fetal weight in each litter was statistically

significantly less that that reported in controls 3.9 ± 0.08 g in the highest dose group compared with 4.1 ± 0.04 g in the control group; p less than 0.05, Mann Whitney U test, two tailed). The author stated that no statistically significant dose-related effects were detected with respect to these observations as well as the other parameters measured.

Discussion and Conclusions:

This study is intended to evaluate a specific effect on the development of the eyes in fetal rats. The data presented by the author supports the stated conclusion that the lowest teratogenic effect level (LEL) is 62.5 mg/kg/day and that under the conditions of the study described herein a no-observed effect level (NOEL) is 30 mg/kg/day.

Core Classification. Supplementary. The study was intended to evaluate a specific effect noted in previous studies.

References

Kavlock R.J., N. Chernoff, L.E. Gray, Jr., J. Gray and D. Whitehouse. 1980.

Report on the teratogenic potential of benomyl administered via the oral and dietary routes in the Wistar rat. Health Effects Research Laboratory. Experimental Biology Division, Development Biology Branch, U.S. EPA, Research Triangle Park, North Carolina.

Staples, R.E. 1980. Benomyl: Teratogenicity in the rat after administration by gavage. Medical Research Project No. 3501-001. Haskell Laboratory Report No. 649-80."

Roger Harden 6-27-83

Roger Gardner
Toxicology Branch
Hazard Evaluation Division
(TS-769C)

* when There are combined with prior date from . Same study, The overall CORE grads

Roger Garian 8-18-53

STUDY TYPE: Teratology - Rabbits

TOX. CHEM. NO.: 75A

HAZLETON LAB. REPORT NUMBER: 210-214 FICHE/MASTER: 00035 MR NO.: 1079 ACCESSION: 091750-6

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Hazleton Lab., Falls Church, Va.

AUTHOR: W.M.Busey

DATE REPORT SUBMITTED: 7/15/68

TEST MATERIAL: Benomyl; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester (50% a.i.)

SYNONYMS: Carbamic Acid, (1-((Eutylamino)-carbonyl)-lH-benzimidazol-2-yl)-methyl ester; (99.15 purity)

INT-1991-99 DPX-3866 N.B. 5103-109

Review by M.L. Quaife, 5/3/71

"We judge following summary to be a fair appraisal of results of study. We add selected values, below (in footnotes), to illustrat findings.

The purpose of this study was to evaluate the potential of fungicide 1991 (Benomyl, Code No. INT-1991-99, powder, approximately 50% active ingredient) for embryotoxic and/or teratogenic effects in (New Zealand White) albino rabbits. The test material was administered in the diet (Purina Rabbit Chow, available ad lib.) at dose levels of 0, 100, and 500 ppm (to 15 each artificially impregnated does/group on days 8-16, of gestation). (Seven or eight does in eachgroup were sacrificed on day 29 or 30 of gestation and the remainder allowed to hutch normally.)†

There were no maternal deaths during the study. One abortion occurred in the low level group. Tissue masses which were apparent fetuses and dead pups were found in the cage pans of one low-level doe and one high-level doe prior to initiation of the treatment period. Both of these animals were sacrificed on Day 6 and were excluded from the study. A total of 34 of 43 does used in this study (excluding the two does which were sacrificed) became pregnant (12 control, 13 low level, and nine high level).

The appearance, behavior, body weight gain*, and food consumptic of the test animals were, in general, comparable to the controls. No evidence of a compound-related effect was noted in the following criteria: Findings from gross necropsies performed on the does; the number and placement of implantation sites, ** resorption sites, ** or live and dead fetuses**** from Ceasarean deliveries; weight and length of fetuses, fetal external appearance, and gross visceral anatomy; the number of live and dead pups from full-term litters, **** pup weight and length, external appearance, and gross visceral

anatomy. The development and structure of test fetal and pup skeletons (studied after alizarin staining and clearing) were comparable with the control animals and with accumulated control data.

Dietary administration of Fungicide 1991 (benomyl) to female albino rabbits from Day 8 through Day 16 of gestation (at 100 or 500 ppm in the diet) had no discernible effect on fetal development.

- * Mean weight gain during 3-week period for controls, 100-, and 500-ppm females is 413, 421 and 369 g, respectively.
- ** Implantation sites, 7.3, 7.3, and 8.0/maternal rabbit--control to high level groups.
- *** Resorption sites, 0.3, 0.9, and 0.2/maternal rabbit (0- to 500-ppm.
- **** Live fetuses, 6.8, 6.0, and 6.8/maternal rabbit and dead fetuses, 0.2, 0.4, and 1.0/maternal rabbit--same progression.
- ***** Live pups, 6.8, 6.0, and 5.5/maternal rabbit and dead pups, 2.0, 0.6, and 0.3/maternal rabbit -- same progression as above."

CONCLUSION: NOEL = 500 ppm (HDT)

CORE-CLASSIFICATION: Supplementary - due to dietary treatment rather than gavage and no maternal or fetal toxicity was evident at the high dose tested.

original review evaluated by M.P.Copley, D.V.M. Tox. Br. 9/18/85

()t added by M.P.Copley to clarify the original review

STUDY TYPE: Teratology - Rats

TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 649-80 M.R.: 3501-001

FICHE/MASTER: ACCESSION: 256575 GS0119-009

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial Medicine, Newark, Del.

AUTHOR: R.E.Staples, J.G.Aftosmis

DATE REPORT SUBMITTED: 9/18/80

TEST MATERIAL: Benomyl; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester; 99.2% a.i.; Lot #71008A.

SYNONYMS: Methyl 1-(butylcarbamoy1)-2-benzimidazole-carbamate
Carbamic Acid, (1-((Butylamino)-carbony1)-1H-benzimidazol2-y1)-methyl ester
INT-1991
DPX-3866

MATERIAL AND METHODS: Pregnant ChR-CR® strain rats, 9 weeks old, weighing from 156.4 to 206.1 gm were obtained from Charles River Breeding Labs, Inc. on days 2 and 3 of gestation (2G, 3G). Day 1G was determined by the presence of sperm in the vagina. They were individually housed, assigned unique animal numbers and fed standard rodent chow and water ad libitum. They were randomly assigned to treatment groups on day 6G such that group mean body weights were similar. Treatment with the test compound* was by gavage from days 7 through 16G. The groups were as follows:

<pre>Dose level (mg/kg/day)</pre>	No. of females
0	60
3.0	27
10.0	27
30.0	27
62.5	27
125.0	27

Observations - Dams were weighed upon arrival and days 6, 7, 11, 16, 18, and 21G (maternal weight gain was calculated for days 7G through 10G) and observed daily.

Sacrifice and necropsy examination - On day 21G, prior to sacrifice by chloroform inhalation, the dams were coded to eliminate bias. The uterus was removed and examined for: number of implantation sites, number and position of live, dead and resorbed fetuses; and number of corpora lutea. The uterus of "non-pregnant" rats was stained with ammonium sulfide to detect very early resorptions.

External alterations - All live and dead fetuses were sexed, weighed and examined for external alterations (2.5x magnification). Visceral alterations - About half of the live fetuses per litter were fixed in Bouins and examined viscerally for soft tissue

^{*} Benomyl suspension was prepared daily and given at a rate of 1 ml/rat/day

alterations using the method of Barrow and Taylor (J. Morph., 127: 291-305(1969). Micro-ophthalmia was determined by measuring the intact globes (eyelids of both eyes removed). Selected eyes were examined microscopically using hematoxylin and eosin stained sections.

Skeletal alterations - The remaining fetuses were prepared for examination 1) eviscerated, 2) fixed in 70 \$ ethanol, 3) macerated in 1 \$ aqueous KOH solution, 4) stained with alizarin red \$. Statistical evaluation - Incidence of pregnancy, maternal death and individual alterations were examined using the Fisher exact test. Maternal body weight and weight gain were tested for significance using a 1 way analysis of variance and Dunnett's test. Jonckheere's test was used to test for dose responses. The remaining fetal parameters were tested using the Mann-Whitney U test. P < 0.05 was considered the level of significance.

RESULTS: There were no overt clinical signs of maternal toxicity. Maternal, reproductive and fetal toxicity - There were no significant differences in: maternal body weight or body weight gain, incidence of pregnancy, incidence of corpora lutea, implantation sites, fetal sex ratio, and stunted fetuses between treated and control groups. Fetuses in the 62.5 and 125.0 mg/kg/day groups were significantly lighter than control fetuses (Table 1).

Teratogenicity -

Malformations - There was a significant increase in malformed fetuses (table II) in both 62.5 and 125.0 mg/kg/day groups due mostly to ocular (microphthalmia and anophthalmia) effects and brain (distended lateral ventricles, hydrocephaly) lesions (high dose only). Fused ribs, sternebrae and arches occurred only at the high dose (see table II). Variations - Several skeletal variations (see table III) including: sternebrai, -hemi, -misaligned, -partially to unossified; and centra, -bipartite, -hemi were significantly increased over controls in the high dose group. Visceral variations were not treatment-related.

DISCUSSION: There was no observed maternal toxicity at any dose; however, Benomyl caused decreased fetal weight at 62.5 mg/kg/day and 125 mg/kg/day. Fetal malformations included hydrocephaly (high dose) and microphthalmia (62.5 and 125.0 mg/kg/day). Haskell laboratories determined their background incidence of microphthalmia between 3/8/76 and 8/20/80 (22 studies). Only 1 case of bilateral anophthalmia and 3 cases of microphthalmia (not litter-mates) were confirmed in 530 litters (with 4,935 fetuses). There were two additional cases of suspected microphthalmia. They concluded that the background incidence for this lesion was 4-6/4935 or 1/1000 fetuses. The study authors speculated that the compound effect on the eye may have also extended to the 10 mg/kg/day dose due to the severity of the microphthalmia in the 2 affected fetuses at this dose and the low background for this lesion. Further studies would be needed to confirm this. There appeared to be

no correlation between microphthalmia and reduced fetal weight.

CONCLUSION: Maternal toxic NOEL > 125 mg/kg/day (HDT)

Fetotoxic NOEL = 30 mg/kg/dayFetotoxic LEL = 62.5 mg/kg/day based on decreased fetal weight

Teratogenic NOEL remains undetermined until the lowest effect level demonstrating increased microphthalmia is determined. A treatment-related increase of microphthalmia was clearly evident at 62.5 and 125 mg/kg/day.

<u>CORE-CLASSIFICATION</u>: <u>Supplementary</u> until the microphthalmia question is resolved.

This study is Core-Minimum when combined with Haskell study number 587-82 which more clearly defines the NOEL and LEL.

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50(83) 6 220.6413.84 21 347.9420.31	26196) 119 9412.11 71.1431.91 90.5416.14		~~	21(65) 216.0211.07 162.2227.00 93.1213.22	21(70) 221.710.10 366.0271.50 34.259.74	**
6 220.6 <u>4</u> 13.04 21 367.9 <u>628.31</u>	90.5416.31		~ ~	91.1413.22	221.7210.18 366.0223.58 34.239.74	**
21 367.9420.31	71.1431.91 90.5416.30 317		~	91.1413.22	36.023.58	**
Body wright gain (418.20.)	30.5416.30			93.1413.22	34.289.74	* ;
	90.5516.36			93. 1413. 22	54.245.24	9
4 :				,		•
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700	2	1). 24). 86	20 20 20		. 7257	
S. E. H. J				12.9£1.48	12.637.26	
athe times dame? 25(18)	201133	71117	70117	14.131	11.721.0m	
P 1.627.61	10, 1415, 44	7.949.10	9.943.17	7.4517.71	15. (123). 26	
		•		•/	•	Land American
The second secon						
	21.7	152	230	213	=	
1, X Mn. / litter(15.0.)	9. 343.61	10.021.74	9.21.33	9. 342. 73	9.082.58	•
. 612	107/108	115/136	115/123	107/106	16/10	:
- b males	20.5	45.0	÷.	50.2	9 . =	
No. stunted	å	-	_	_	•	Ň.
1. Telal selubil(418.9.)	4.140.17	4. 220. 25	4.150.31	3.640.31	3.540.51	``
						•
			Ì			
duse been on everage body weight on thay 6 of gestation	50					

b females with visible sign of pre-mancy evident at sutopay duse besed on sverage body weight on Day 6 of gestation

C identified as having been prognant only by somenius suifide staining of the uterus, data from these fomales used only for calculation of "total 2 prognant" d includes data only from females with visible sign of prequancy at autopsy

c atunted and dead fatuses were excluded, expressed as everage of mean fring weight/litter

* nignificant done-response se determined by Josekhenre's test

* nignificantly different from control incidence by two-tailed Monachiliney W test



BENOMYL: TERATOGENICITY STUDY IN THE RAT WHEN CIVEN BY GAVAGE FROM DAYS 7 THROUGH 16 OF GESTATION

FETAL MALFORMATIONS

		واستعصب				
				g/kg/day	· · · · · ·	
	Control	1.0	10.0	30,0	02.5	125,0
<pre>gaternal No. examined =fetuses/litters No. maiformed =fetuses/litters</pre>	1/1	217/26 b	251/24	238/26 1/1	213/23	181/20 6/4
<pre>visceralc,d No. examined "fetuses/litters No. malformed "fetuses/litters</pre>	237/50 3/3	102/23	120/24	113/25 ^e 1/1	101/22 ^e 12/0	89/20 23/9
skeletal ^d No. examined =fetuses/litters No. maiformed =fetuses/litters	258749 ⁸ 171	115/26	131/24	125/26	112/23	92/20 4/4
Total no. malformed =fetuses/litters	5/5		2/2	1/1	12.6	26/9
Avg. % malformed fetumes per litter(-5.0.)	0.9±3.2	0.0	0.7-2.3	0.4-2.0	5.1 <u>~</u> 13.5*	19.0-3204
titters	i					
External Cleft palate Hydrocephaly Edema Multiple maiformations [©] Hicrognathia Upturned snout	1/1	b		1/1		2/2 1/1 3/2 2/2
Visceral ^C +Microphthalmis/anophthalmis +Lateral ventricles - distended Hydronephrosis	1/1		2/2	·	10/4 ^h 2/2	21/8. 6/3 1 i
Skeletal Nasal and premaxilla-short Sternebrae-fused Ribs-fused Arches-cervical-spread -thoracic-fused -cervical-reduced number Centra-fused	1/1					1/1 1/1 0 1/1 1/1 2/2 1/1

⁴ dose based on average body weight on Day 6 of gestation

blanks represent zero incidence of affected fetuses

c includes alterations detected in the heads of the fetuses examined for visceral alterations; in the high dose group also includes 2 fetuses with microphthalmia that were examined skeletally

in any fetus includes only selformations at sizes other than those detected externally

difference from no. litters examined externally because one or more litters consisted of only one fetus which was examined for only visceral or skeletal alterations

¹⁰⁰ X g _ Mo. malformed fetuses in the litter _____ /total no. of litters

F # 257992, f - edema lower neck and jaw, gastroschisis, meningocele, bilateral microphthalmia, 1 digit missing and 1 curved, laterally displaced lower limbs and tail, 3 chambered heart, fused pulmonary artery and sortic arch

h 7 of these 10 fetuses were from 1 litter (F # 258027)

significantly different from control incidence by two-tailed Mann-Whitney U test

significant dose-response as determined by Jonckheere's test

BENOMYL: TERATOGENICITY STUDY IN THE RAT WHEN GIVEN BY GAVAGE FROM DAYS 7 THROUGH 16 OF GESTATION

	PETAL VARIA	TIONS		• .	: .	٠٧.
	Control	3.0	10.0	g/kg/day ^a	62.5	125.0
resal no. fetuees with vertations/litters	293/50	130/25	135/24	140/25	118/22	102/18
Avg. 1 (etuses with variations/litter	61 (<u>+</u> 20.4)	61 (<u>+</u> 26.0)	53 (<u>+</u> 21.4)	57 (<u>+</u> 25.4)	53 (<u>+</u> 20.7)	52 (<u>+</u> 22,7)
No. affected fetuees/no. affected litters External						
Hematoms Petechise	23/18 12/11 -	15/10	14/8 7/5	17/14 2/2	8/7 8/7	9/9 5/4
Visceral Liver -peliosis		1/1	1/1			
Lens -email pocket Renal papilla-reduced Renal pelvis-enlarged	6/5 91/34	2/2 37/13 1/1	44/15	33/14 2/2	33/15	1/1
Skeletal Skull partially ossified					-	
-interparietal -parietal	1/1	5/3	1/1			
-suraccipital -squamosal	1/1 1/1	1/1 2/2	,	1/1	1/1	
Sternebrae d		1/1			2/2	5/3**
-bipartite -misaligned (2°)	1/1	4/3	1/1		1/1 .	2/2 11/9**
-misslighed (1)* -unossified -partially unossified	6/5 17/10 86/38	5/4 11/5 31/15	3/3 9/5 32/15	2/2 3/5 47/19	4/4 12/6 36/16	5/4, 11/8, 53/17
Ribs	*****		30,13	47,17		
-missing ⁶ -uavy -calloused		1/1		1/1	1/1	1/1
-partially ossified -extra ossification sile(s) ^h -rudimentary	1/1 112/ -5 19/12	51/20 6/6	48/20 6/4	50/17 18/12	40/17 11/8	39/17 12/7
-extra	4/3	1/1	1/1	4/2	5/3	4/3
Centra -dumbbelled -bipartite.	1=/11 3/3	6/5 3/3	9/7 2/2	9/7 3/3	10/9 8/7	15/8 16/9#:
-fused -heri -missligned -unossified -pertially ossified	1/1				1/1	3/3 ⁴ 1/1 2/1 1/1
-displaced Arch -partially ossified					1/1	1/1 2/2

a dose based on average body weight on Day 6 of gestation

the number of fetuses examined were identical to that listed in Table II except that fetuses with malformations were excluded

¹⁰⁰ X I No. malformed fetuses in the litter /total no. of litters

d variations in sternebra V were excluded

at less: two sternebra with lateral halves misaligned by one-third or more of their length

same as for (a) except only one sternebra missligned

f did not appear to be due to technical error

the presence of a fourteenth rib as a round or ovel center of ossification

the fourteenth rib was termed rudimentary if its length exceeded twice its width

the fourteenth rib was termed extra if its length was greater than or equal to the length of the preceding rib

significant dose-response as determined by Jonckheere's test

significantly different from control incidence by two-tailed Mann-Whitney U test

Significantly different from control incidence by Mann-Whitney U test but only by one-tailed test

significant dose-response as determined by Cochran-Armitage test

significantly different from control incidence by Fisher's exact test

MR100081913

004679

STUDY TYPE: Two-year Feeding study-Dog

TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NO: 48-70

FICHE/MASTER: 00097305

129-69 53-71 00081913

54-71 74-77 00097**318** 00097**32**5 00061**618**

MR NO.: 966

SPONSOR. E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: haskell Lab. for Toxicology and Industrial

Medicine, Wilmington, Del.

AUTHORS: h.Sherman, J.R.Barns, E.F.Stula, G.J.Stopps

DATE REPORT SUBMITTED: 3/17/70, supp. path. report 11/22/77

TEST MATERIAL: 1-butylcarbamoy1-2-benzimidazolecarbamic acid,

methyl ester (approx. 50 % a.i.)

SYNONYMS: benomyl

INT-1991

Banlate@wettable powder

Review by Bruce Jaeger in the 1983 WHO report:

"Groups of beagle dogs (four males and females/group) one to two years of age, were administered benomyl (50% active ingredient) in the diet at dosage levels of 0, 100, 500 and 2500* ppm for 2 years. Food consumption and body weight data were obtained weekly, and animals were examined daily for clinical signs of toxicity. Hematological, biochemical and urinalysis examinations were performed periodically throughout the study. Interim sacrifice after one year was performed on one male and one female from control and high dose groups. Organ weights, gross necropsy and histopathological evaluations were performed at the conclusion of the study. Only the livers and testes were examined histologically in the 100 and 500 ppm dose groups.

There was no mortality related to treatment. Body weight changes and food consumption values were similar among all groups, except the high dose which demonstrated both decreased food intake and body weight gain. The average daily dose was 55-58 mg/kg body wt. (initially, M & F), 74-79 mg/kg (at one year) and 45-55 mg/kg (at 2 year). One dog at the high dose lost its appetite and was replaced. No other clinical signs of toxicity were observed. Hematological evaluations and urinalyses were similar to control. Males in the 2500 ppm group had increased cholesterol, alkaline phosphatase and GPT values (initially), as well as decreased total protein and albumin/globulin (A/G) ratio. There were similar, but less

see addendur for clarification and additional information.

marked effects in high dose females. Cholesterol and total protein were similar to controls among the females examined.

The biochemical determinations were supportive of adver-

The biochemical determinations were supportive of adverse liver effects, demonstrated as liver cirrhosis among high dose group animals. There was also slight to marked bile duct proliferation in 4/6 dogs at the 2500 ppm level. Hemosiderosis, evident in one dog in the 2500 ppm group at one year, was not evidence in other dogs examined at 2 years after staining specifically for iron. Preparation of preserved wet tissue with oil red 0 and sudan black for hepatocyte vacuolation confirmed that benomy! was not hepatotoxic at 100 and 500 ppm in the diet."

Addendum:

MATERIAL AND METHODS: Food was offered ad libitum between 3:00 PM and 7:00 AM; there was free access to water. Hematological tests, biochemical tests and urinalysis were performed 3 times pretest, and 1, 2, 6, 9, 12, 15, 18, 21 and 24 months after test initiation.

Hematology - Red blood cell count, hemoglobin, hematocrit, total and differential reukocyte counts.

<u>Biochemistry</u> - Glucose, urea-nitrogen, cholesterol, alkaline phosphatase (APase), glutamic-pyruvic transaminase activity (GPT) total protein (TP), and albumin/globulin ratio (A/G).

<u>Urinalysis</u> - pH, volume, osmolality, protein, sugar, urobiling acetone, bilirubin, occult blood and microscopic sediment examina Tissues from the control and 2500 ppm groups were fixed in Bouin' and stained with hematoxylin and eosin for histologic examination

included:

tadrenal	mammary gland	tonsil
prostate	esophagus	trachea
tpitultary	cecum	qa!l bladder
pancreas	colon	spinal cord
urinary bladder	trachea	salivary gland
epididymis	skeletal m.	, 3
Fallopian tubes	peripheral n.	
uterus	bone marrow	
ovary	eye	
duodenum	thoracic aorta	
	prostate tpitultary pancreas urinary bladder epididymis Fallopian tubes uterus ovary	prostate esophagus tpitultary cecum pancreas colon urinary bladder trachea epididymis skeletal m. Fallopian tubes peripheral n. uterus bone marrow ovary eye

RESULTS:

KESOFIS:									
			MALE	S (PPM)	1	FEMAL	ES (PPM)
		0	100	50 <u>0</u>	2500	0	100	500	250 0
Chol.(2	mon.)	125	121	106	182	151	147	132	145
(2	yr)	116	128	111	166	161_	141	135	179
APase(2	mon.)	2.0	1.9	1.2	8.9	2.5	1.5	1.9	2.6
(2	yr)	1.6	1.8	1.3	4.0	4.2	0.9	0.9	1.8
GPT (2	mon.)	22	19	22	163	18	70	21	39
(2	yr)	23	20	23	23	21	16	14	29
Alb/G(2	mon.)	.80	.80	.91	.72	.99	1.20	0.92	0.88
(2	yr)	•98	1.02	1.11	.81	1.14	0.92	0.96	0.77
Tot. (2	mon.)	5.80	6.64	5.78	5.57		no tr	eatment	relat
Prot.(2	yr)	6.32	6.36	6.27	5.75		chang	e	

Increases in (male) chol. and APase started as early as 1 month and remained elevated throughout most of the study in the 2500 ppm group. GPT (male) increased by 1 month but returned to normal levels within the 15 months in the 2500 ppm group. Alb/G ratios (males) decreased within 2 months and remained low throughout the study in the high dose group. Total protein (male) was slightly decreased within 1 month and remained low throughout the study in the high dose. Correlation coefficient analysis of chol., APase, GPT, Alb/G and total protein indicated a relation between the level of compound in the food and the change in blood levels. An F test also indicated differences between the treatment groups for these parameters (2 standard deviations were considered significant). Hepatic cirrhosis was evident grossly and microscopically in one male sacrificed at 1 year and 2 males and 1 female sacrificed at 2 years.

Sacrifice time	lyr	2 yr	
Incidence of hepatic	1/2 male	2/3	male
cirrhosis	0/1 female	1/3	female

Focal testicular degeneration was present in all treatment groups, with marked testicular degeneration (reduced testes weight, absence of spermatozoa and spermatic giant cells) in 1/3 dogs at 2500 ppm.

DISCUSSION: The report indicates that treatment was temporarily witheld from 2 male dogs that lost weight (high dose). One of these was off the compound for weeks at a time. There was no mention of frequency or duration of these occurrences. Although individual body weights were presented, there were no summaries present in the report which made weight gain difficult to analyze. Hepatic cirrhosis observed in the high dose males and females was probably treatment related. As reported by the previous reviewer and the registrant, the histologic and biochemical changes indicate possible liver damage in males at 2500 ppm. An outbreak of an inflammatory disease causing orchitis in beagle colonies at that time may have contributed to the unusually high level testicular lesions in the controls and treated groups. K. Davis, D.V.M. (Tox. Br. pathologist) of EPA examined the testes slides (see report of April 30, 1971) and concluded that "Certainly neither the degree or the distribution indicate that testicular changes are related to chemical ingestion." Data from the 2 year dog study on carbendazim, a primary metabolite of benomyl, provide additional confirmatory results for the absence of testicular effects at dietary levels of at least 100 ppm carbendazim. There were no other treatment related changes evident in this study.

Although the previous reviewer (WHO review) listed the NOEL as 100 ppm, the data supports a NOEL of 500 ppm and a LEL of 2500 ppm based on hepatic cirrhosis and clinical chemistry alterations.

CONCLUSION:

NOEL = 500 ppm

LEL = 2500 ppm based on biochemical and histological alterations indicating liver damage as well as decreased weight gain and food consumption.

CORE-CLASSIFICATION: minimum

Original review evaluated and addendum added by M.P.Copley, D.V.M.

Tox. Br. 9/19/85

Reviewed by: Marion P. Topley, D.V.M. Section 6, Tox. Branch (TS-769C) Secondary reviewer: Jane Harris, Ph.D. Section 6, Tox. Branch (TS-769C)

gett 10/1.185

004679

DATA EVALUATION REPORT

STUDY TYPE: Mutagenesis - micronucleus

TOX. CHEM. NO.:

75

ACCESSION NUMBER: (GS01\$9-008)

TEST MATERIAL: MBC, Benomy!

JOURNAL ARTICLE: Mutation Research, 40 (1976) 339-348

TITLE OF REPORT: The mutagenicity of benzimidazole and benzimidazole derivatives. VI. Cytogenetic effects of benzimidazole derivatives in the bone marrow of the mouse and the Chinese hamster.

AUTHOR(S): J.P.Seiler

REPORT ISSUED: 1976

CONCLUSION: author's conclusions for micronucleus formation:

MBC (mice) NOEL = 50 mg/kg LEL = 100 mg/kg

Benomy1 (mice) NOEL = 500 mg/kg LEL = 1000 mg/kg

serum concentration of MBC NOEL = 8 ug/l (as long as 24 hr)
LEL = 11.5 ug/l (for less than 6 hr)

Classification: incomplete because the above mentioned conclusions could not be confirmed or rejected with the available data.

MATERIALS: Benzimidazole, MBC, 2-benzimidazolylurea, benzimadozolecarbamonitril, 2-aminobenzimidazole, benomyl

Animals: ICR mice, 8 wk old, 25-30 gm

METHODS: Micronucleus test: Material was administered by i.p. (intraperitoneal) or be gavage (in 2% gum arabic) twice, 24 hr apart. The mice were sacrificed 30 hr after the first treatment. Bone marrow smears were prepared for micronucleus and metaphase examination. See table I from the article for treatment summary and results. Serum levels of [2-14C]MBC were measured at various times after single treatment with the labelled compound. See table IV from the article for treatment summary and results.

RESULTS and DISCUSSION: MBC, administered i.p. appeared to be insoluble as evidenced by a mass of MBC in the peritoneal cavity and constant serum levels regardless of dose or time (see table III). There was an increase in micronuclei both polychromatic and normochromatic. The author examined Chinese hamster bone marrow smears after gavage administration of

1000 mg/kg MBC for chromosome breakage. Due to negative results, he concluded that the micronucleus formation was due to interference with the mitotic process rather than chromosome breaks. He felt this was supported by an earlier onset of action with MBC (micronucleus formation as early as 6-8 hr) than with the known alkylator, trenimon (16 hr).

with the known alkylator, trenimon (16 hr).

The author suggested there may be a threshold limit (of 11.5 ug/l MBC in the serum) for spindle inhibition and that MBC is 10 times more potent for this effect than benomyl. However, there is no mention of number of replicates or animals/dose, no indication as to sample variation or whether gum arabic was given to the control group in table 1. The methods and results were presented in abbreviated and vague terms. For the above reasons the authors conclusions could not be confirmed or rejected with the available data.

ABLE!

ICRO NUCLEATED ERYTHROCYTES (PER 1000 POLYCHROMATIC ERYTHROCYTES) FROM

OUSE BONE MARROW AFTER TREATMENT WITH SEVERAL BENZIMIDAZOLE COMPOUNDS

٠,

empound	Permula	Appli- cation	Amount ms/ks	Micronucleated polychromatic crythrocytes	Micronucleated normochromatic erythrocytes
ensimi-		i.p.	100	4.3 3.1	2.8 2.6
(BC	NH COOCH3	i.p. p.o.	\$00 \$0 100 \$00	4.8 4.2 13.4 19.7 26.8	1.8 2.9 6.5 12.3 18.2
l-Benrusi- dazolylurra	MH CONH2	, p.e.	\$00 1000	7.2 16.3	3.7 8.0
Benzimida- zoletarba- monitril	CL NHCH	9.0.	500	3.5	2.3
2-Amino- benkuni- darole	NNH2	9.0.	100 800 1000	3.2 4.1 2.8	3.0 1.9 2.5
Benomyl	NH COOCH3	p.o.	500 1000	4.2 12.6 —	3.0
None (renti	CO NHC4H.			2.6	2.5

TABLE IV

 $(3.14\,\mathrm{G})$ MeC in blood serum of Mice treated orally or intraperitoneally (radio-activity counts per ML serum)

The specific activity of the MBC suspension was 1.15 µCl/mg.

reatment	Amount (mg/kg)	Time after treatment (b)	sbw - 10 ₋₃	MBC conc. #E/ml serum
J.O.	100	4	29	11.5 -
		6	20	Ĭ.
	800	2	42	17
		.	61	24.5' " -
		•	53	.31 .
			34	13.5
		16	24	9.5
		24	27	11
P.	100	4	20	8
•		6	. 19	7.5
Þ.	\$00	2	20	8
		4	19	7.5
		6	20	8
		. 8	21	8.5
	•	16	22	•
	**	34	21	8.5

Reviewed by: Marion P. Copley, D.V.M.

004679

Section 6 , Tox. Branch (TS-769C)
Secondary reviewer: Jane Harris, Ph.D. 944 /0/10/85
Section 6 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Metabolism

TOX. CHEM. NO.: 75A

ACCESSION NUMBER: 091561-F

MRID NO .: 00066776

TEST MATERIAL: Benomy!

STUDY NUMBER(S): none given

SPONSOR: E.I. DuPont De Nemours and Co., Inc.

TESTING FACILITY: not specified

TITLE OF REPORT: Metabolism of Methyl 1-(Butylcarbamoyl)-

2-C-14*Benzimidazolecarbamate

AUTHOR(S): JA Gariner, H. Sherman, RW Reiser

REPORT ISSUED: not specified (1968?)

CONCLUSION: The major urinary metabolites appear to be conjugates

of 5-OH-MBC.

Classification: unacceptable, only 1 male rat was tested

MATERIALS: compound - Benomyl, Methyl 1-(Butylcarbamoyl)-Benzimidazolecarbamate with and without label

animals - rat, male, ChR-CD, 172 gm at start

METHODS: A single rat was treated with 2500 ppm unlabeled benomy! in the food (1 % corn oil was added). After 12 days (weight of the rat was 264 gm) the rat was given 7.7 mg of benomy!-2¹⁴C by gavage and placed in a glass metabolism cage. The system contained a trap for converting organic volatiles to CO₂ and a CO₂ trap. Urine and feces were collected daily for 72 hours. After 3 days, blood was drawn by cardiac puncture, the animal sacrificed and the following tissues removed for radiolabel analysis: brain, lungs, heart, liver, spleen, kidney, testes, gastrointestinal tract muscle, fat and the carcass.

RESULTS: The rat weighed 268 grams at sacrifice. Table 1 (from the report) indicates the percent of recovered radioactivity in the various organs and excreta. At 72 hours less than 1% remained in the carcass, with 86 % of recovered radioactivity found in the urine and 13 % in the feces. TLC analysis of the urine initially indicated 2 spots (1 major and 2 minor). After enzyme hydrolysis however only 1 spot accounting for 80 % of the radioactivity on the plate, remained. Its R_f value differered from the other three. This indicated that glucuronide

and/or sulfate conjugates were excreted in the urine. Very little, if any parent or MBC was present in the urine. The spot had the same $R_{\rm f}$ as 5-hydroxy-methyl-benzimidazolecarbamate (5-OH-MBC). Its identity was confirmed by mass spectroscopic analysis.

DISCUSSION: This study is limited because only one rat (male) was tested using pretreatment. Females may have a different metabolic pattern. Pretreatment may have resulted in more rapid metabolism of the parent. This study would need to be compared to single dose studies in order to develop a clearer understanding of the metabolism of benomy!

When the control of the interpolation
When the control of th

	Percent of	Rynoverad Ra	ರ್ಷಿತ್ರದಲ್ಲಿಗೆ
Protection of The and and	?: '	i? her.	700
carbon dioxida	₩.D.²	W.D.	W.D.
rine	78.9	85.3	85. ?
Iaces	3.7	12.2	13.1
Noid mash of cage			0.4
7112			2.2
2000			
ileed			< 0.11
TTÅA			< 0.31
Tab	4	e.	< 0.01
;.i. tract			0.2
eer:			< 0.01
:iineyo			< 0
iver			(0.2
ungs			`< 0.5%
118 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1			< 0.31
iplean			< 0.01
.estes			< 0.01
ercess (less fur)			0.02
•	87.6	97.5	100

^{*}Over-all recovery was 91.5% of administered C14-activity.

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²N.D. - None datected.

Data Evaluation Report

004679

Compound Benomyl (Benolate® 50WP)

Citation
2-14 C-Benomyl (50% WP) absorption through rat skin
Part II: Effect of time and dose, I.J. Belsco, Biochemicals
Dept, Research Div, Experimental Sta, E. I. du Pont de
Nemours & Co Inc, Wlimington, Delaware, 19898, March 9. 1979.

Reviewed by Robert P. Zendzian PhD Pharmacologist

Core Classification Acceptable

Conclusion

Benomyl was absorbed through the rat skin from Benolate 50WP in small amounts in a nonlinear dose and duration related manner. Percent absorption ranged from 0.031 (high dose) to 3.518 (low dose) for the maximum exposure of 10 hours.

Materials

[2- ¹⁴ C] Benomyl as 50% wettable powder (Benolate® Fungicide)
Formulation #1 1.642 uCi/mg
Formulation #2 0.162 uCi/mg

Eighty male ChR-CD rats, 224 - 250 gm.

Methods

"Treatment Rates

- (1) 0.2 mg ¹⁴C-Benø ate•/rat Formulation 1
- (2) 2 mg ¹⁴C-Benolate ●/rat Formulation 1
- (3) 20 mg 14C-Ben#1ate*/rat Formulation 2
- (4) 200 mg 14C-Ben#1ate*/rat Formulation 2

Exposure time for each treatment was 0.5, 1, 2, 4 and 10 hours. Four animals were used at each treatment level and time interval."

The back of each rat was clipped and the clipped area washed with acetone 24 hours before treatment. Doses 1 and 2 were applied by syringe as water suspensions and doses 3 and 4 by spatula as a thin paste. Exposure area was 4 in² (approximately 16% of the rat's surface area). Applied material was spread with a Teflon® rod. 'Application' devices were counted for loss of dose. Rats were placed in individual collection containers and urine and feces collected sepentately. Blood samples were taken from all

004.7

animals at termination. All samples were assayed for total benomyl.

In addition urine samples were assayed qualititively for benomyl metabolites.

Results

Table A. Benomyl in the Blood. a) Mean equivalent concentrations of benomyl (ppm or ug/ml). b) Mean quantity (ug) assuming 6.4% blood volume. Data from tables 1, 2, 3, and 4 of the report.

Dose	*	Durat	on of e	xposure	(hours)	
mg/r	at	0.5	1.0	2.0	4.0	10
0.2	a b	0.001 0.016	0.004	0.004 0.064	0.004 0.041	0.003 yrm = p./~
2.0	a b	0.006 0.096	0.009	0.008 0.128	0.008 0.128	0.004 0.064
20	a b	0.026 0.416	0.028 0.448	0.034 0.544	0.036 0.576	0.024 0.384
200	a b	0.033 0.528	0.054 0.864	0.048 0.768	0.070 1.120	0.064 1.020

^{*} Bendiate 50% WP

Table B Mean equivalent total amounts of benomyl in the urine (ug). Data from tables 1, 2, 3, and 4 of the report.

Dose*	Durati	on of ex	posure (hours)		
mg/rat	0.5	1.0	2.0_	4.0	10	
0.2	0.03 (.030)	0.11 (.110)	0.65 (.650)	1.67 (1.60)	3.47 (3.470)	16 das que je
2.0	0.07 (.008)	0.31 (.032)	1.05 (.106)	3.27 (.328)	-4.85	,
20	0.23 (.002)		3.29 (.032)	4.03 (.040)	9.27 (.092)	
200	0.40 (.001)	3.62 (.004)	8.79 (.008)	28.55 (.028)	30.33 (.030)	

^{*} Benoilate 50% WP (percent of dose of active ingredient)

Table C Mean equivalent total amounts (ug) and percent of dose of benomyl in urine and blood. Sum of data from tables A and B $\,$

Dose*	Durati	on of exp	osure (ho	urs)	
mg/rat	0.5	1.0	2.0	4.0	10
0.2	0.046 (0.046)	0.174 (0.174)	0.714 (0.714)	1.734 (1.734)	3.518 #5 (3.518)*/dec
2.0	0.166 (0.016)	0.454 (0.045)	1.178 (0.118)	3.398 (0.340)	4.914 (0.491)
20	0.645 (0.006)	1.108 (0.011)	3.924 (0.039)	4.606 (0.046)	9.624 (0.096)
200	0.928 (0.001)	4.484 (0.004)	9.549 (0.095) , .0/ 0 .	28.670 (0.029)	31.359 (0.031)

"Thin-layer chromatographic analysis of an extract of the composite urine from the 2 and 200 mg treatments for 10 hours revealed the presence of 5-HBC as the major benomy! metabolite with a lesser amount of MBC as shown in radio scan and autoradiogram. ---- No 4-HBC was detected in these samples."

Discussion

This study does not provide data on the amount of compound which new have been retained in the rat at termination of a particular exposure. However, the Gardiner et. al (1968) study of benomyl metabolism in the rat provides information indicating that any retained material would be insignificant. Following a single oral dose of 29 mg/kg (7.7 mg/rat) the post absorption phase equlibrium t1/2 has been graphically determined to be 10.5. Under the circumstances of this dermal absorption study such a half-time should not lead to significant bioaccumulation of the compound. The dermal absorption generated in this study may be used for evaluating dermal exposure/dose of benomyl.

Quantity and percent of each dose that was absorbed increased with duration of exposure but the increase was not linear with time. For example, a ten fold increase in duration of exposure, from 1 to 10 hours, resulted in 20.6, 11.0, 8.8 and 7.0 fold increases in the quantities absorbed per respective dose. A five fold increase in duration of exposure, from 2 to 10 hours, resulted in 4.6, 4.2, 2.5 and 3.2 fold increases in the quantities absorbed per respective dose.

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For each unit time of exposure the quantity of material absorbed increased with increasing dose but the percent of the dose absorbed decreased with increasing dose. This relationship was also not linear.

These dose/time relationships are expected with the majority of compounds which are absorbed through the skin.

Reference

Gardiner, J.A., Sherman, H. & Reiser, R.W. (1968?) Metabolism of Methyl 1-(Butylcarbamoyl)-2-C-14* Benzimidazolecarbamate in the Rat. E.I. du Pont.

d by: Marion P. Copley. V.M.
6, Tox. Branch (TS-76)
ry reviewer: Jane Harris, Ph.D.
6, Tox. Branch (TS-769C)

CJ4371

DATA EVALUATION REPORT

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STUDY TYPE:

TOX. CHEM. NO.: 75A

- 1) Teratology mouse gavage
- 2) Teratology rat gavage
- 3) Teratology rat dietary
- 4) Postnatal rat gavage (dams)

ACCESSION NUMBER: (GS0119-017)

TEST MATERIAL: Benomyl

JOURNAL ARTICLE: Toxicology and Applied Pharmacology 62,44-54(1982)
(HERL - USEPA: 1/11/80)

TITLE OF REPORT: Teratogenic effects of benomyl in the Wistar rat and CD-1 mouse, with emphasis on the route af administration

AUTHOR(S): RJ Kavlock, N Chernoft, LE Gray, Jr., JA Gray, and D Whitehouse

REPORT ISSUED: 1982

CONCLUSION:

 Teratology - mouse - gavage teratogenic NOEL = 50 mg/kg
 LEL = 100 mg/kg (supra occipital scars, subnormal vertebral centrum, supernumerary ribs and cleft palate)

CORE-CLASSIFICATION: minimal

2) Teratology - rat - gavage
 teratogenic NOEL = 31.2/mg/kg
 LEL = 62.5 mg/kg (microphthalmia and increased fetal
 mortality, reduced fetal weight)

CORE-CLASSIFICATION: minimal

3) Teratology - rat - dietary teratogenic NOEL > 500 mg/kg (HDT) (approx. 6760 ppm*) toxic. NOEL = 169 mg/kg (approx. 1690 ppm*) LEL = 298 mg/kg (approx. 3380 ppm*)(weight decrease in fetuses)

CORE-CLASSIFICATION: minimal

4) Postnatal - rat - gavage (dams), fetotoxic NOEL = 31-2 mg/kg | 5.6 fetotoxic LEL = 62.5 mg/kg (decreased weight of testes, ventral prostate, and saminal vesicles)

CORE-CLASSIFICATION: supplementary

SEE ATTACHED ARTICLE

^{*}time weighted averages for dietary concentration

Emphasia on the Route of Administration

ROBERT J. KAVLOCK, NEIL CHERNOFF, L. EARL GRAY, JR., JACQUELINE A. GRAY, AND DOUGLAS WHITEHOUSE

Experimental Biology Division, Health Effects Research Laboratory, U.S. Embrana Research Triangle Park, North Comban 27711

Received March 77, 1961, accepted September 1, 1961

Terringuis Effects of Basemyl in the Wistor Ret and CD-1 Mones, well Emphase so the Renes of Administrator & Locate, J. A., Commonder, N., Gart, L. E., E., G. Gart, J. A., and WirtTHOUGH, D. (1981). Tosted Appl. Pharmond & 42, 44-34 Basemyl, a system fragicist when makestale being of soited Appl. Pharmond & 42, 44-34 Basemyl, a system interest device operation with the device operation of soil and the system of the sys

grasses, and roses (Federal Register, December 6, 1977). It was developed in the late 1960's as one of the first evistemic fungicides. Benomyl ((methyl 1-butylcarbemoyl)-2-benzimidazole carbamate) is a fungicide registered for fruits, auts, vegetables, rice, belbs, flowers, ornamental crops, shade trees,

The paper has been review... by the Health Effects Agency, Landerstory, U.S. Environmental Presistion Agency, and appeared for publication. Mention of tradements or conservate products from not constitute to Sand request for reprints to Dr. Robert J. Karbott, Dresistonestal Binkey, Branch, Experimental Binkey, Dresistonestal Binkey, Branch, Experimental Binkey, Person, Sprinners, U.S. Environmental Protection Agency, Research Telescope Park, N.C. 27711.

Since that time, it has become widely used in both the field and the home (Beat, 1979). It offsetiveness against fungal growth in attributed to its ability to interfere with mitosis (Hammerschäng and Sieler, 1973), specifically by binding to tubulin (Davidue, 1973; Davidue and Flach, 1977), and then preventing tubulin polymerization. White besonnyl and its breakdown product in aqueous medium (methyl-2-heazinindazod carbamata, or MBC) (Käigore and White, 1970) do effectively bind to fungal tributin and prevent misionis, both have relatively low affauity for mammanism tubulin to be and Flach, 1977; Friedman and Platzer, 1978; Ireiand et al., 1979; and do not readily

contained account out that nor measurement to be be desired to these components designed their effective fungicidal action. The oral LDS0 in solids rate in excess of 10,000 mg/kg (Sciler, 1973). Approximately 90% of a possible state of the solid section of the Despite for toxicity to adult mammade, several authors have continued that due to the brochemical basis of action, benzimido-

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the components may pear a genetic risk to mad (Sciler, 1975; Bignami et al., 1977; it Durinks and Fach, 1977), and exponent of rats to both becamp! and MBC has been linked with damage to the reproductive system. Administration of beneary to Watstern's as on greated in increased incidences of recorded crokeys and malderned feterors of recorded crokeys and preser (Shenn-berg and Torcheshy, 1972). Amanakas included crossopholms, bydroccybaly, and marchylablance, the incidences were determined. The authors reported to office on feturose expend to 0.25 mg/lagfory. Torcheshy, or of (1976) observed changes in sestivities biochemistry and histology, spering motificial biochemistry and histology, spering motificial biochemistry and histology, spering motified to the complete and protecting and protecting and protecting and protecting and protecting and protecting and complete activities and protecting and prot doze (31.5 mg/kg/day), but testicular suc-cinate dehydrogenes and corebellar accep-cholineaterace activities were depressed. No effects were observed in the lowest doze icsted (6.25 mg/kg/day). Delatour and activity is male Water rats receiving personal administrators of benomy 1st 500 mg/kg/day for up to 11 months. No himselegical changes were observed at the next lowest Richard (1976), in a starty of the conbryo-

not greece increased the incohence of re-norption, decreased fittal weight, and pro-doctal transit assembles were not detailed, but included connecipally, bydeverghely, microphilahimi, bygelysphania of the lineal, farinn of vertebral bedies, citil palese, and hydeverghelami, Comizzay to those cape-dective effects of benearly and MRE (allow-ing as administration, Sherman or ed., (1873) found no effect of dictary administration of 2500 ppm becomes the Sprague-Duelky rest in a three-generation reproduction study or ed 5000 ppm in a terrodogy study. This lat-ter dictary level was approximately 400 mg/ kg/day, or more than three times the effecive dose noted by Shorsburg and Terchinsky (1972) in po-dened Wister rats. The present studies were industed to forther evaluate the effect of reuse of administration of becomy on enabyseric development in the rat. We also examined the effect of oral administra-tion of benomy! in a second species, the not affect embryonic development, admin-sitration of MBC at done of 19.1 mg/kg LATIONCY CHES DIS LADYS & NO 13 ON BESTAINED ON

METHODS

Yorkeand grots beneated supplied by E. I. Duffeet Softwares and Company (Willemgers, Data.) was seed.

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ERATOGENIC EHILLIS OF BLICATIC

Band and Tag Co., Newport, Ky.). Mice were housed for get eagle and 1sts were boused indevidually on plat-shaving bedding in polypeopyliste capes. They received tap water and Parine Lab Chow and Hikitum.

Mouse Prematal Study

Benomy) was administered via garage as a suppension in a crapped corn and (Lot D-4.3). Essimana facility Co., Rechester, N.Y.) as a rate of 0.1 ml/mesma-fdsy. The suspansions were prepared delity with the sid of a magnetic stierer Doess were 200, 100, 30, and 9 mg/hg/mit stierer Doess were 200, 100, 30, and 9 mg/hg/mit stierer Doess were 200, 100, 30, and 9 mg/hg/mit stierer Doess were 200, 100, 30, and so magnetic stierer bear series of mg/hg/mit stierer and may be material weight. Control aminals received the whole sides. Animals were randomly assigned to treatment groups and treated on Duys 7 to

Another term is that by decapitation on Day 18 of gratition.

Animals were is littled by decapitation on Day 18 of an animals were it is the by decapitation on Day 18 of animals were it is the service of the grand at the meternal weight gain of prognancy has the weight of it he grand unters. The maternal was removed and weight of the grand unters. The maternal was removed and weight of the grand unters. The maternal was removed and weight of the grand of the grand of the service of the grand of the service of the service of the service were the removed with the service of the service

Ret Prenated Studies

Benomy was administered to the down other in the date of yarge, After belacing on the basis of Dry 4 material weight, rats were trademly amigned to treatment groups such that the mean and varience of

is the general control of the control of the property of the general control of the control of t Day 4 weight were the same for each treat

Stettitical Ambyres

Ret Pretmetal Study

Basemy) was administered we prouge as described above. The date levels were 31 2, 15 6, and 0 mg/hg/

mg/kg/day group were different from control values. While values for fetures in the lowest done group were generally in line with the done reapones, no paired comparisons with control values were againficantly different. It is noteworthy that all classes of supernamerary lumbar ribs were affected by treatment. The percentage of lumbar ribs in increasing order of done were: extra: 24, 8.4, 15.5, and 17.5, small: 17, 6.9, 16.7, and 13.8; and very small: 6.4, 12.6, 9.6, Only a few parameters for fetuses in the 100 and 22.7. day. The down were treated from Day 7 of gestation considered by 8 of inclusion (Day 22 of gestation was considered Day 8 of inclusion). At bettle, the page were considered canonical for external defects, apparented by an included to the forest of the facts of the page were related to the more than eight page, with a opposite an arrive as possible. The litera were than weighted to bay it in the considered and the inclusion to possible. The litera were the weighted to Day 8, 15, 22, 24 (1977) was determined at 15, 28, and 100 days of age in a figure-capt mass of the conclusion of the lost accrety measurement, the conclusion of the lost accrety measurement and the ventral products plus accessed by the accretion of the control products plus accessed to the control products of page of the control products and page of the control products accessed to the control products of the control products accessed to the control products accessed to the control of the control products accessed to the control of the control

Major anomalies observed in mice fetuses exposed to benomy! during organogenesis are presented in Table 5. The overall inci-dence was 1.3, 1.0, 16.8, and 47.3% at 0, 50, 100, and 200 mg/kg/day, respectively.

Rats

change in the highest-dose group and probably resulted from reduced litter size due to Oral teratology study. Administration of up to 125 mg/kg/day of benomyl during organogenesis did not affect food consump-tion but did affect maternal weight change after constion of treatment (Table 2). This effect was limited to a decrease in weight Data are presented as the mean place or mores the a standard error of the mean. The later was regarded as it the experimental town of comparison for all analysis, a carpit data recorded for personaling animals where the individual was the said of comparisons. Treatment effects over determined by Their's caracter of for quan-tal data and by ANOVA for anappeared state When a sugardizant treatment effect was determined by AN-OVA, Denoted and subspheriousy test was aspined for peared comparisons. All official are reported sequences at the p < 0.05 keed. Oral teratology study. Administration of

RESULTS

increased fetal recorption in that group.

The effects of benomyl on the maternal organism and fetal development are presented in Table 3. No treatment effects were evident either on maternal viability or on maternal weight gain. There was a significant cant treatment-related effect on the incidence of embryonic recorptions, although it was individually significant from the control value only at the highest-dose level. Six fitters in the high-dosage group were com-pletely resorbed. Fetal weight was signifi-eastly affected by the administration of benomyl, and fetures from the donage groups above 31.2 mg/kg/day weighod significantly ten than control fetures. Indicators of stelmaturity (the developmental score for up to 200 mg/kg/day of benomyl during Days 7 to 17 of gestation did not affect macternal viability of growth (Table 1). Benomyl did, however, exert adverse influence en upon fetal development. Embryonic viability, fetal weight, skeletal maturity (supraoccipital ossification), docreased nambers of disternal and caudal ossifications, delayed dooccurrence of supernumerary ribs were all a subversely affected by benomyl exposure. The responses were dose related. All these features for fetuses in the high-dose group were significantly different from control values.

relopment of the vertebral centrams, viaceral maturity (incidence of enlarged cerebral ventricles and enlarged renal pelves), and the

EFFECTS OF ORAL ADMINISTRATION OF BENCHMY, ON FETAL DEVELOPMENT IN MICE

KAVLOCK ET AL TABLE

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		Dem	Dum (mg/lg/dey)	
Oherration	0	2	901	900
Maternal				
No trested	2	ສ	2	2
Ye dead	•	•	•	-
No pregnant	2	2	=	=
Ne reserbed (Icris)	-	•	•	-
Weight gain (g)	34 1 03	65 2 04	70 : 89	65 2 84
No implentations	121 1 05	119 1 06	103 1 07	10 1 61
Fetal				
Mortaluy (%)	88 1 4%	107 ± 2F	_	217 : 68
Wenght (g)	100 1 001	100 : 00	0 14 1 000	872 · 96F
Seprency print score	13 2 81		_	24 · 07
No sternebrae	50 × 01	57 4 01	-	20 . 05
No coudal vertebrae	70 × 04	70 1 17	_	27 + 05
Sebnormal vertebral centrams				
ê	*		-	751 1 9 9
Separaumerary ribs (%)	105 ± 5 F	***	410 2 615	20 = 75
Enlarged lateral ventrubes (%)	•		•	232 : 87
Enlarged renal petres (%)	•			337 . 66

Note A t SE, means with same superscript are not supadicianly different, p < 0.05

centers, and the occurence of subnormally ossified vertebral centrums) and visceral maturity (the occurrence of enlarged cere-bral ventricles) were adversely affected by benomyl treatment and reflect the effect ossification of the supraocciptal, the groups were compared with control values, it was evident that these effects were not upon fetal weight. When individual dosage number of sternal and caudal osufication observed at dotages at or below 31.2 mg/

Major anomalies present in fetuess exposed to benoary! by no administration are presented in Table 5. The mailconnation rate in the control group was approximately i. 4%. In the lowest two design groups (15.6 and 31.2 mg/kg/day), the incidence of mail-formations was 3.1 and 5.2% of the total fetues, respectively. Approximately 19% of the fetues from the 62.5 mg/kg/day group in Mad a detectable abnormality. At 125 mg/s

abnormality, the most prominent being en-cephalocekes, hydrocephaly, clefts of the lip or jaw, gastroschisis, and fused vertebrae. kg/day, 35% of the fetuses had a detectable

aive to the dame, but only the 6760 ppm dict was aversive during the latter two sampling periods. As a result of this reduction in food consumption, maternal weight change dur-ing organogenesis was adversely affected during Days 7 to 10 of gestation. The re-duction in weight gam continued for dams Dertary teratology study. Dietary admin-istration of benomy! during organogenesis realted in significantly reduced food con-numption at all monitoring periods (Table 2). Pair-rise comparisons between control and treated groups indicated that initially both the 3340 and 6760 ppm diets were averin the high-duage group during Days 10 to 14. During the last measurement period (between the time of return to control diet and sacrifice of the dams), dams in the high-

dotage group reversed the previous weight	gave and increased their body weight signif-	scaetly more than did controls. Tame-	resignate averages of actual excitary concentration and food consumption indicated that	neimels received approximately 169, 298,	and 505 mg/hg/day in the low-, middle-,	and high-distary concentration groups, re-	spectively.	in the streets of entitory expenses to benowly and fact demonstrate one presented in Table	4 A sireificant effect was observed on me.	The second secon	ternel weignt gast caring gentleton. Only	chans in the highest doesge group had weight	changes individually deferred from the con-	į	cidence of fetal mortality was observed. Fe-	tal weight was significantly affected by	treatment, with the effect individually sig-	mificant from the control value for fetures in	both the 3340 and 6760-ppm dosage groups.	The only indicators of sheletal or vioceral	maturity affected by treatment were the os-	sisting of the supraorcipital base (with a	irol dosage groups) and the presence of ca-	larged renal pelves (with the effect most	promisent in the two highest duage groups).	No dese-related incidences of anomalies		from dome in the dictary regimen (I able 5).	CONTRACT MARRON	you accommend to rate during and alice and	lactation are presented in Table 6. Benomyl	died not affect litter size or weight at birth,	and growth and sarvivability of the pups to	y wearing. At birth, two pape with sacmalics	were found in the 15.6 mg/kg/day dunage	groups (one with a short tail, one with an- asarca). No activity changes due to treat-	-	in a higher-eight make at 15, 24, or 100 days of age. A permanent treatment-related re-	doction in the wagnet of the costst and the ventral proteins and scaninal ventrals was ventral and an analysis of the costs as 5% reduction in the manufacturing and it is reduction.	
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ENTIRETS OF ADMINISTRATION OF BENOMYL TO WISTAR RATS ON FOOD CONSU

dictary administration. In the present stor

oral administration of benomyl produc

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EFFECTS OF ORAL ADMINISTRATION OF DENOMY, ON FETAL DEVELOPMENT IN RATS

TABLE 3

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MAJOR ABIQUAMATITIS IN ROBERT FITARES EXPOSES TO BENCHALL

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TERATOGENE EFFECT OF MITTO FILE

TABLE S

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in the weight of the ventral prostate and seminal vesicles for animals in the 31.2 mg/kg/day dosage group. No other treatment-re-

day dosage group. No other treatment-re-lated differences in organ weights were ev-ident.

DISCUSSION

The studies reported are in agreement with both the work of Shtenberg and Torchinsky (1972), who reported on the tera-

TABLE 4

EFFICTS OF DISTARY ADMINISTRATION OF BENDAVE ON FETAL DEVELONMENT IN RATS

		Burn ingen to dea	1	
Observation	•	3	Ī	**
1	×	2	=	R
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	40 - 44	10. 4.43	110 . 11	
		115 . 40	11 - 41	•
			82 0 28	::
		1	74.04	
Catalog and part (%)				

T. C.K.

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each experiment can be accomplished by calculating the regression of fetal weight on dose for the three cases and then determinregressions are: gavage rat, fotal weight =-(0.00680) (dose) + 3.7399; dectary rat fotal weight = -(0.000753) (dose) + 3.3058 inserved by gavage to the rat, in the duc dose) + 1.0089. Therefore, 55, 512, and 7 g the douage secessary to effect a 10 g/kg/day are necessary to produce a 10 perved in all three experimental groups (gr novesate in fetal weight. The calculate Since fetal growth retardation was rage rat, dietary rat, and gavage mo comparison of the potency of beno

to the rat, and by gavage in the mouse, respectively. By using a similar approach and was observed in rat fetues from down re-criving 6760 ppm (approximately 505 mg/ kg/day) benomyl in the diet. togenic activity of benomyl following administration via oral gavage, and the work of Sherman et al. (1975), who reported on gross terata at doses above 31.2 mg/kg/day in the rat and 50 mg/kg/day in the mouse. The defects observed involved a broad range sex glands. Only fetal growth retardation the lack of teratogenic activity following of tissues and were consistent with the action of microtubule inhibitors (Tamaki et al.

postation resulted in a permanent reductiv

in the size of the testes and male acces 31.2 mg/kg/day benomyl to rats throu

1966). Furthermore, oral administration

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TABLES

EFFECTS OF ORAL ADMINISTRATION OF BENCHITY, BUSING THE PRESENTAL PERIOD ON PUSTNATAL DEVELOPMENT IN RATE (# # 3E)

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Open suges, Day 100 (A)	71 5 5 (8)		,	•	Ė		
3	W	125 . 64163	,	,	31.10		
Refers (g) Oreres (mg)	=======================================	151 060 183	1	1 1	5 B		
Tenna (mg)	MA - 1023		1574 t 48 (34)		18 : BIN		
(Bar) eresmad	mm : 10 (120		1912 4 45 (34)		- TOTAL - 1 1884		
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cally, be expected to occur at a dietary exposure level of 663 mg/kg/day. Then, in the rat, the dietary route of administration appears to be an order of magnitude less potent than the gavage route of administration.

Other examples of alterations in teratorbe seen that since malformations occur in the rat after gavage at a does that produces at 15% reduction in fetal veright (6.2.5 mg/ kg/day) a similar veright reduction (and therefore malformations) would, theoretiassuming the slopes of the dose-response curves are similar for fetal growth retarda-tion and for the induction of terata, it can

ture. Dipterex is teratogenic in the rat when genic potency subecquent to afterations in route of administration exist in the literaadministered in the diet, but not when given

pound three times per day by gavage dad produce malformations in the rat (Staples and Goulding, 1979). Similarly, EDTA is day so injection or twice per day by gavage (Kimmel, 1977). a more effective teratogen when administered in the diet as compared with once per once per day by gavage (Staples et al. 1976). However, administration of the com-

In view of the factors that regulate the amount of substance reaching the fetur, it is not surprising that large differences in terquestion to ask is which route of exposure, if any, is the more appropriate one from which to extrapolate results to the human atogenic response would result from differing rowles of administration. The important situation. Fundamental differences in con-

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And Surcessor, Q. W. (1979) Permand seasory of outbook in addition 1. Principle and the seasons of neutral part of permand seasory of outbook in addition 1. Principle and the seasons of the season of the porally concentrated pattern. White some food is consensed desiring the finance land of the light dark cycle, most food consemption occurs in short boars throughout the actional phase (Borber) and Huston, 1974). Ther, given equal potential for metabolism of a chemical by get microflors and equal potential for metabolism potential for absorption into the blood, decistry administration of chemicals with short budgated half-lives such as becomy! would sammatory behavior exist between the spe-cies commonly used in teratology biossays and bumans. Feeding behavior in the labo-

appropriate desing regimes when expected homes exposure is through indirect contam-ination of the food, and risk extrapolations should be based on this regimen.

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We thank Dr. D. Broad and C. Tots of the Ann-lyneal Chemistry Broads, Emmanued Tournings Driven, for analysis of beneaty broats.

ACKNOWLEDGMENTS

DRAIM, M. ALKEND, F. VHCKE, A. CARRER,

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be expected to result in low circulating levels

in the bloodstream, as exercisen per unit time can equal or exceed ingestion per unit time. Humans, on the other hand, consume the bulk of their found at two or three discrete time persons during the day. Such tempo-rally concestrated feeding behavior would be expected to result in higher peak plasma levels of short-fixed chemicals administered

in the duet than would occur in the rat. To

the authors, this difference in feeding be-

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Secondary reviewers:

Reviewed by: Dynamac . Wennerberg, W. McLellan, 1.C. Felkner 034679 Marion P. Copiey, D.V.M. Marion P. Copiey, D. Copies, D.

DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity - mice

TOX. CHEM. NO.: 75A

ACCESSION NUMBER: 246948A, 246949,246950

MRID NO .: 00096514

TEST MATERIAL: Benomy!

SYNONYMS: Methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate

STUDY NUMBER: Haskell No. 20-80

SPONSOR: E. I. du Pont de Nemours and Company

TESTING FACILITY: Haskell Lab. for Toxicology and Industrial

Medicine, Newark, Del.

TITLE OF REPORT: Long-term feeding study with Methyl-1-

(butylcarbamoy!)-2-benzimidazolecarbamate (INT-1991, Benomy!,

Benlate®) in mice.

AUTHOR(S): P.W. Schneider, Jr., B.E. Wiechman, T. Dilworth; et al.

REPORT ISSUED: Jan. 26, 1982

CONCLUSION: NOEL for carcinogenicity < 500 ppm (LDT)

Carcinogenic at 500 ppm (LDT):

hepatocellular adenoma and carcinoma in males and fem

pulmonary alveologenic carcinomas in males,

Degenerative changes in the testes and epididymides

at 5000-7500 ppm (HDT)

Classification: Core-minimum

MATERIALS: Benomyl, 99-99.2% pure, lot #s INT-1991-366, INT-1991-4:

grey crystalline material.

SEE ATTACHED REVIEW

CONFIDENTIAL BUSINESS ESTORMATION DOES NOT CULTIMIN MATIONAL SECURITY INFORMATION (EO 12045)

004679

EPA: 68-01-6561 TASK: 81 June 13, 1985

DATA EVALUATION RECORD

BENOMYL

Oncogenicity in Mice

CITATION: Schneider, P.W., Jr.; Wiechman, B.E.; Dilworth, T.; et al. Long-term feeding study with methyl l-(butylcarbamoyl)-2-benzimidazole-carbamate, (INT-1991, Benomyl, Benlate®) in mice. (Unpublished study, Report No. 20-82 by Haskell Laboratory for E.I. Du Pont De Nemours & Co., Inc., Wilmington, DE; dated January 26, 1982.)

REVIEWED BY: Paul Wennerberg, D.V.M., M.S. Signature: Project Scientist Dynamac Corporation Date: William L. McLellan, Ph.D. Senior Scientist Dynamac Corporation I. Cecil Felkner, Ph.D. Signature: Program Manager Dynamac Corporation Date: 6-17-85 APPROVED BY: Marion Copley, D.V.M., M.S. Signature: **EPA Scientist** Date: Jane Harris, Ph.D. Signature: **EPA** Section Head Date:

DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity in mice.

CITATION: Schneider, P.W., Jr.; Wiechman, B.E.; Dilworth, T.; et al. Long-term feeding study with methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate, (INT-1991, Benomyl, Benlate®) in mice. (Unpublished study, Report No. 20-82 by Haskell Laboratory for E.I. Du Pont De Nemours & Co., Inc., Wilmington, DE; dated January 26, 1982.)

ACCESSION NUMBER: 246948-A, 246949, 246950.

MRID NUMBER: 00096514.

<u>LABORATORY</u>: Haskell Laboratory for Toxicology and Industrial Medicine, Elkton Road, Newark, Delaware 19711.

<u>QUALITY ASSURANCE STATEMENT</u>: Chronological summary present and signed but not dated.

<u>TEST MATERIAL</u>: Methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate was supplied in two lots (INT-1991-366 and INT-1991-414) as a grey crystalline material which was stated to be 99% and 99.2% pure, respectively. It was used to prepare test diets from 8-29-78 to 9-9-80. Throughout the study, INT-1991 was refrigerated until used.

PROCEDURES:

- Three hundred and twenty male and 320 female 4 week old CD®-1 mice were used from Charles River Breeding Laboratories, Wilmington, Massachusetts. After a thirteen day acclimation period, they were divided using computerized stratification to randomize by sex into four groups of 80 animals per sex, each group having approximately equal mean body weights. The mice were caged individually in stainless steel wire-mesh cages.
- 2. Diets were freshly prepared each week and stored under refrigeration until used. Ground Purina Laboratory Chow diet was mixed with test compound in corn oil to achieve the following concentrations: 0, 500 ppm, 1,500 ppm, 7,500 ppm. After 37 weeks on the diet, the highest concentration, 7,500 ppm, was reduced to 5,000 ppm. All diets contained 1% (w/w) Mazola® Corn Oil. Throughout the study, all mice received the appropriate test diet and tapwater ad libitum. Samples

of diet containing test material were collected for analysis during the following times: 1) at the time of preparation; 2) after storage at room temperature for 24 hours and 7 days; 3) after storage under refrigeration for 7 days. These samples were collected four times during the study and analyses showed no degradation of test compound. Data for test diet homogeneity were not presented.

- 3. All mice were examined daily for clinical signs of toxicity and palpated at least once every two weeks for tissue masses. Mice were weighed weekly (weeks 1-26), biweekly (weeks 26-52) and monthly (weeks 52-104). Recorded during the same times were body weight gains, food consumption, food efficiency and intake of test compound. Mortality was also recorded.
- 4. Ten mice per sex, per group had hematological examinations at intervals of approximately 1, 3, 6, 12, 18, and 24 months after the start of the study. The following parameters were examined: RBC, WBC, and differential WBC counts, hemoglobin, hematocrit, total plasma protein, MCV, MCH, and MCHC. Blood smears were prepared from all surviving mice at study termination.
- 5. Gross necropsy was performed on all mice used in the study regardless of time of death. Organ weights and relative organ weights (per final body weights) were obtained from all animals at terminal sacrifice for the following organs: brain, heart, lungs, liver (with gallbladder), spleen, kidneys (with adrenals attached), testes (with epididymides), and thymus. All Guideline-required organs except the rectum were examined histologically by "conventional methods."
- 6. The following statistical procedures were performed by the study authors: body weight and organ weight data were analyzed by one-way ANOVA. Hematological data were analyzed by crossed and tested ANOVA. The least significant difference or Dunnett's test was used to analyze differences between treatment groups. Survival was subjected to Kaplan Meier methods¹. Comparisons of survival distributions and tumor incidences were analyzed by the Mantel-Haenszel method². Comparisons of absolute proportion of survival and incidences of tumors and clinical observations were analyzed by Fisher's Exact test. Dose responses in tumor incidence were analyzed by the chisquare test for trends. The level of statistical significance was p < 0.05.

¹ Kaplan, F.L., and Meier, P. 1958. Nonparametric estimation for incomplete observations, Journal of the American Statistical Association, Vol. 53, 457-481. (reference not presented by authors)

Mantel, N. and Haenszel, W. 1959. Statistical aspects of the analysis of data from retrospective studies of disease, Journal of the National Cancer Institute, Vol. 22, No. 4, 719-748. (reference not presented by authors)

Unless otherwise noted, the word "significant" in this review has statistical connotations (p < 0.05).

RESULTS:

<u>Clinical Observations and Mortality</u>: No clinical observations in any treatment group were reported to be significantly different from controls. Individual and summary data showed that there was no increase in the number of treated animals with palpable masses as compared to controls.

Body Weight and Food Consumption: Table 1 presents mean body weight data for male and female mice at selected intervals during the study. Both male and female high-dose mice showed a significant reduction in mean body weight throughout the course of the study. The mid-dose groups showed a significant reduction in mean weights at 60% of the weighing intervals for males (32/53) and 40% for the females (21/53) when compared to controls. There were only 2 instances of significant weight reduction in both male and female low-dose groups. Mean body weight gains showed significant decreases from controls in about 50% of the mid- and high-dose male weights and about 25% of the mid- and high-dose female weights. Food consumption was slightly decreased in males and females at the mid- and high-dose groups compared to controls; however, statistical analyses of the data were not provided and could not be validated by our reviewers without individual data.

Hematology: According to the report, there were no dose-related alterations in hematologic parameters. Mean hematocrit, erythrocyte count, and hemoglobin concentration were slightly but significantly lower in mid-dose males than in controls from months 3-24. A very slight but significant decrease in erythrocyte count and increase in mean corpuscular volume and mean corpuscular hemoglobin concentration observed from months 3 to 24 in females receiving the high dose of benomy! were not considered compound related when compared with controls. Mid-dose females also showed a significant increase in the mean corpuscular volume and a significant decrease in the mean corpuscular hemoglobin concentration.

Organ Weights: There were significant increases in mean liver weight in mid-dose males and in liver-to-body weight ratios in mid- and high-dose males and in high-dose females when compared to controls (see Table 2). Brain-to-body weight ratios were significantly increased in low- and high-dose males and in high-dose females. Mean testes weight was significantly lower in high-dose males than in controls and kidney weights were significantly lower in high-dose females than in controls. Thymus weights were decreased in all dosed males when compared to controls. The increased liver weights and decreased testes weights were correlated with histo-pathological changes, and considered of biological significance by the authors. The other changes in organ weights were considered to be of equivocal biological significance in the absence of a dose-related trend and histopathological changes.

TABLE 1. Mean Body Weights of Mice Fed Benomyl for 104 Weeks At Selected Time Intervals.

<u>Mean Body Weight (gm)</u> Week						
Group/Dose (ppm)	0	13	56	80	104	
Males						
0	26.6	38.2	47.1	47.7	43.5	
500	26.6	38.9	47.6	46.6	42.5	
1500	26.6	37.3*	45.7	45.6	41.2*	
5000-7500ª	26.5	34.4*	42.3*	42.4*	39.7*	
Females						
0	21.0	30.3	37.8	38.9	36.5	
500	21.0	30.3	37.2	38.0	34.0	
1500	21.0	30.1	36.8	36.6*	35.7	
5000-7500a	21.0	27.9*	33.4*	34.3*	33.4*	

 $[\]star$ Significantly different from controls value (p < 0.05) when analyzed by ANOVA by study authors.

 $^{^{\}mathbf{a}}$ Reduced from 7500 to 5000 ppm after week 37.

Selected ${\bf d}$ Mean Absolute and Relative Organ Weights at Terminal Sacrifice from Mice Fed Benomyl for 104 Weeks \cdot TABLE 2.

•				שונו			
Group/Dose (ppm)	Body Weight	Liver	Thymus	Testes	Brain ^c	Liver	Thymus
Control	44.35	2.58	0.0	0.43	1.14	5.86	0.16
200	42.30	2.64	0.05*	0.41	1.21*	6.26	0.12*
1500	42.13	3.29*	0.05*	0.44	1.19	7.80*	0.13*
2000-7500b	40.34*	3.06	0.05*	0.38*	1.24*	7.54*	0.14
				FEMALES			
					Relative		
	Body Weight	Brain	Kidney	Brain	Liver	Thymus	
Control	38.54	0.48	0.69	1.26	5.39	0.15	
200	36.30	0.48	0.64	1.35	5.67	0.15	
1500	37.25	0.50*	0.67	1.37*	6.14	0.18	
2000-7500	34.44*	0.48	0.62*	1.40	7.08*	0.19*	

 $^{\rm d}$ (*) Significantly different from control value (p < 0.05) when analyzed by study authors. $^{\rm d}$ 7500 ppm changed to 5000 ppm after week 37. $^{\rm c}$ Organ:body weight ratio.

<u>Gross Pathology</u>: Individual animal gross necropsy findings were reported but summary data with statistical analysis were not provided nor were the gross findings discussed by the authors.

Histopathology: Significant incidences of non-tumor histopathological changes are presented in Table 3. Tissues of dosed animals showing significantly increased incidence of lesions as compared to controls were: thymus in males at 5,000 ppm (atrophy), thymus in females at 1,500 ppm (cysts), liver in males at 5,000 ppm (5 parameters showing hepatocellular alteration), spleen in females at 5,000 ppm (hemosiderosis), trachea in females at 1,500 and 5,000 ppm (lymphocytic infiltrates in the submucosa), testes in males at 500 and 5,000 ppm (atrophy and tubule degeneration), epididymides in males at 5,000 ppm (aspermia), prostate in males at 5,000 ppm (focal distended acini), thyroid in males at 500 and 5,000 ppm (distended colloid follicles), and nasal cavity in males at 5,000 ppm (interstitial fibrosis and amyloidosis).

Significant incidences of neoplastic changes are presented in Table 4. In the males, the incidences of hepatocellular carcinomas, combined hepatocellular adenomas and carcinomas, and pulmonary alveologenic carcinomas in the 500 and 1,500 ppm groups were significantly higher than controls. In the females, the incidences of hepatocellular carcinomas in the 500 and 5,000 ppm groups and combined adenomas and carcinomas in the 1,500 and 5,000 ppm groups were significantly higher than controls. The same five parameters showed a significant trend (p < 0.05) when analyzed by our reviewers using the Cochran-Armitage Trend test.

The mean-time-to-, and median-day-of-tumor discovery were stated by the study authors not to be significantly different between treated and control groups. Individual animal data (in the form of time to death with tumors present) were provided.

DISCUSSION:

The authors concluded that benomyl, fed at a minimum of 500 ppm, produced a significant increase in hepatocellular carcinomas in male and female mice. There was a significant dose response to treatment in females for hepatocellular carcinomas and combined hepatocellular neoplasms. Our review of the study substantiated these conclusions; however, several conclusions were not supported.

When we reanalyzed the data, we found several significant compound or treatment effects that were not discussed by the authors. There was a significant dose-related trend in the incidence of male pulmonary alveologenic carcinomas, hepatocellular carcinomas, and combined hepatocellular neoplasms in males. There was also a significant histopathological dose-response effect in male epididymides and thyroid. When the mean-time-to, and median-days-of-death, with lung alveolar cell carcinomas present, were analyzed by these reviewers using Kruskal-Wallis ANOVA, p < 0.05, all male treated groups were significantly lower than control (Table 5).

TABLE 3. Selected[®] Incidences of Non-Meoplastic Histopathologic Lesions in Mice Fed Benomy1 for 104 Weeks

				Dose Level (pom)				
•			Male	5000-			Fema e	2 4 4 4 4
Tissue	0	500	1500	7500b	0	500	1500	5000 7500
Thyraus	(58)¢	(40)	(38)	(48)	(62)	(62)	(52)	(57)
-etrophy -cyst	1	. 6.	. 5.	12*	5	4	9*	1
Liver -foci of hepatocellular	(77)	(80)	(79)	(80)				
alteration -karyomegaly and cytome-	. 1	3	2	1.				
galy -foci of ceroid, micro-	9	5	12	21*				
granuloma -foci of hepatocellular	55	26	35	38*				
ballooning, degenerati -lymphocytic foci/inflam)-	1	0	6*				
matory inflitrates	38	48	45	52*				
Spleen -hemosiderosis					(76)	(79) 5	(78) 6	(74) 7*
Trachea -lymphocytic infiltrates submucosa	•				(77) 0	(79) 0	(78) 7•	(77) 6*
Testes	(78)	(79)	(79)	(79)				-
-degenerated semini- 'erous tubules tive seminiferous	10	19	15	27*				
tubule degeneration	7	17-	10	17*				
-etrophy -interstitial cell	12	12	8	31*				
hyperplasia	4	4	7	18=				
Epididymides -espermia -distended tubules/	(78) 18	(78) 11	(79) 12	(79) 30°				
tubules filled with degenerated sperm	9	5	11	174				
Prostate —distended acini, focal	(73) 1	(73)	(76) 0	(77) 7#				
Thyroid -distended colloid	(65)	(74)	(73)	(71)				
follicles	4	13*	6	18**				
Mesal cavity	(72)	(68)	(71)	(69)				
-interstitial fibrosis and amyloidosis	1	0	2	7=				

^{4 (*)} Significantly different from control value (p < 0.05) when analyzed by study authors. b 7500 ppm changed to 5000 ppm after week 37. c No. of animals examined. d No data entry signifies a non-significant finding. e Significant trend (p < 0.05) using Cochran – Armitage trend test by our reviewers.

TABLE 4. Selected Incidences of Neoplasams in Mice Fed Benomy! for 104 Weeks

	Dose Level (ppm)							
	Male			Female				
Tissue	0	500	1500	5000 ^b 7500	0	500	1500	5000- 7500
Liver	(77)°	(80)	(79)	(80)	(77)	(80)	(79)	(77)
-hepatocellular adenoma -hepatocellular	9 .	9	11	10	2	2	7	7
carcinoma -combined adenomas and	16	26*	41*	17 ^d	2	7*	6	14ed
carcinomas	25	35*	52*	27 ^d	4	9	13*	21#d
ung	(79)	(79)	(79)	(80)	(77)	(79)	(78)	(74)
-elveologenic carcinome	13	24*	23*	164	16	7	4	6

^a (#) Significantly different from control value (p < 0.05) when analyzed by study authors. ^b 7500 ppm changed to 5000 ppm after week 37. ^c No. of animals examined. ^d Significant trend (p < 0.05) using Cochran—Armitage Trend test by our reviewers.

TABLE 5. Mean-Time-to, and Median-Day-of Death, When Lung Alveolar Cell Carcinomas were Present in Rats Fed Benomyl for 104 Weeks

Dose	Day	'S	
(ppm)	Male	Fema le	
0	736.8 ^a 23.4 743	674.0 101.9 740	
500	665.4* 94.1 728	674.4 84.6 715	
1500	688.9* 96.6 741	719.5 33.0 736	
5000-7500 ^b	702.2* 54.9 739	730.7 11.3 737	

 $^{^{\}rm a}$ Upper value is the mean, the middle value is the standard deviation, the bottom value is the median-day-of-death.

 $^{^{\}mathrm{b}}$ 7500 ppm changed to 5000 ppm after week 37.

Significantly different from control (p < 0.05) when analyzed by these reviewers using Kruskal-Wallis ANOVA.

The mean weight gain over the course of the study was significantly decreased for mid- and high-dose males (14.3 and 13.3 g, respectively as compared to 17.1 g for controls) and high-dose females (12.5 g as compared to 15.5 g for controls), when analyzed by ANCOVA, p < 0.05. Statistical analyses for mean daily food consumption, food efficiency and daily intake of benomyl were not reported and individual animal data were not available, hence, these data could not be statistically analyzed by our reviewers. The summary data provided by the authors showed either no change from controls or a slight compound-related decrease. The latter was especially true for the high-dose female daily mean food consumption with a lesser decrease for high-dose male daily mean food consumption.

The administration of test compound caused no statistically significant increase in mortality in dosed animals when compared to controls at 78, 91, and 103 weeks of the study. At terminal sacrifice (105-106 weeks), the mid-dose female group had significantly fewer animals alive (23 (29%) vs 33 (41%) for control), but the low- and high-dose groups equaled the control value. The total number per group per sex for "found dead" or "moribund sacrifice" were not significantly different from controls except for the female mice found dead. The low-, mid-, and high-dose values were significantly greater (10/80, 12/80, and 11/80 respectively), than the control (2/80) when we analyzed the data using the Fisher exact test.

The authors stated that the hematologic changes were not of biological significance. However, the authors used a method of statistical analysis of the hemotological data that they did not adequately describe; therefore, the analyses could not be reproduced. The findings by the study authors however, allow a clinical diagnosis of toxicological importance when the authors' following significant findings are combined in the high-dose (5,000 ppm) females: 1) hemosiderosis in the spleen, 2) decreased red blood cell counts, 3) increased mean corpuscular volume, 4) increased mean corpuscular hemoglobin, 5) hepatocellular alterations (neoplasms). This information is indicative of regenerative hemolytic anemia. Using the more traditionally employed methods (Bartlett's test for homogeneous variance followed by ANOVA or Kruskal-Wallis test depending on whether a parametric or non-parametric test was appropriate) we found that the only significant hemotological parameter to change from controls was mean corpuscular hemoglobin values in the high-dose females.

The majority of the significant non-tumorous histopathological observations were not considered by the author to be compound related. Our assessment is that several of the changes are commonly seen in aged rats, however, the occurrence in only the high-dose group may imply a compound-related effect.

There were two reporting deficiencies. The clinical observation summary table provided for alopecia/dermatitis (the most prominent observation) was slightly under-reported when compared with the individual animal data. When we reanalyzed this data, none of these parameters were found to be significantly different from controls.

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When we summarized and statistically analyzed individual animal necropsy data, no compound-related effect was seen with respect to the number of masses or nodules when treated groups were compared to controls. The number of masses seen at gross necropsy were about 30% of the number seen histologically.

Our criticisms of this study do not alter the general conclusions of the authors that under the study conditions, benomyl was carcinogenic at the lowest dose tested. There were no additional major deficiencies in the study.

CONCLUSIONS:

Under the conditions of this study, benomyl fed at a minimum of 500 ppm was carcinogenic in the liver and lung of CD-1 mice. Hepatocellular carcinomas were induced in both males (low and mid doses) and females (low and high doses). The combined incidence of hepatocellular adenomas and carcinomas were statistically increased in the mid- and high-dose females. Pulmonary alveologenic carcinomas were induced in males at the low and mid dose. The testes and epididymides showed degenerative changes at the highest dose tested.

CORE CLASSIFICATION: Minimum.

STUDY TYPE: 90 day feeding study - Dogs TOX. CHEM. NO.:

HASKELL LAB. REPORT NUMBER: 283-70 FICHE/MASTER: 00099130

MR NO.: 1270

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial

Medicine, Wilmington, Del.

AUTHORS: H. Sherman, K.S. Carrol, C.W.Eddy

DATE REPORT SUBMITTED: 1970

TEST MATERIAL: 2-benzimidazolecarbamic acid, methyl ester; 50 % wettable powder (53% tech.), (metabolite of Benomyl)

SYNONYMS: MBC

INE-965

MATERIAL AND METHODS: One year old beagle dogs were given food and water ad libitum (between 4 pm-7 am), observed daily for behavior and weighed weekly for a month prior to test initiation. During this period, blood and urine samples were checked for the parameters listed in the lab. test section. Four males and 4 females were randomly assigned to each of the following treatment groups:

Group	Treatment (based on %a.i.)
Control (I)	food
Low dose (II)(LDT)	food 100 ppm MBC (.01 %)
Mid dose (III)(MDT)	food 500 ppm MBC (.05 %)
High dose (IV)(HDT)	food 1500 ppm MBC (.15 %)**
**Lowered from 2500 ppm	due to weight loss

Diets were prepared weekly. The HDT group was gradually given increasing amounts of MBC using the following schedule: 500 ppm - 3 days; 1000 ppm - 2 days; 1500 ppm - 2 days; 2500 ppm for a short time before the dose was lowered to 1500 ppm (week 3) due to decreased food consumption and weight loss.

Observations - Animals were observed daily for toxic signs, mortality and behavior throughout the study.

Body weight and Food consumption - Animals were weighed and food consumption measured weekly.

Laboratory tests - The following tests were done three times during the pretest period and again after 30, 60 and 90 days of treatment.

Hematology - red blood cell count, white blood cell counts
(total and differential), hemoglobin conc. and
hematocrit.

Urinalysis - Urine vol. (24 hr), osmolality, protein, sugar, acetone, bilirubin, appearance, color, pH, presence of occult blood and microscopic examination for sediment.

Clinical chemistries - Glucose, urea nitrogen, cholesterol, alkaline phosphatase (AP), glutamic-pyruvic transaminase activity (GPT), total protein and albumin/globulin (A/G) ration

Sacrifice - All dogs were euthanized by electrocution after 90-105 days of continuous feeding and were examined for gross and microscop changes. Tissues were fixed in Bouin's solution and stained with Haskell quadrichrome. The following organs were removed for weight, fixation and staining: brain, heart, lungs, liver, spleen, pancreas, kidney, testis, prostate, stomach, thyroid, adrenal, thymus and pituitary. The following additional tissues were removed for fixation and staining: ovary, epididymis, Fallopian tubes, uterus, urinary bladder, duodenum, ileum, jejunum, cecum, colon, rectum, muscle, sciatic nerve, bone marrow, eye, aorta, mammary gland, gall bladder, spinal cord, trache, salivary gland, lymph node and skin. All tissues in the control and HDT were examined microscopically however only the liver, kidney and testes were examined at the LDT and MDT.

RESULTS: All animals survived the treatment period. Body weight in the high dose males decreased about 6.8% while all other groups, male and female, gained weight similar to the controls. Food consumption was decreased when the MBC was 2500 ppm, however it returned to control values after the level was lowered to 1500 ppm. The ave. daily dose in mg/kg of MBC received by the dogs was:

		females
100 ppm	2.7	2.7
500 ppm		11.3
1500/2500 ppm	40.7	35.0

There were no treatment related <u>clinical signs</u> or changes in <u>hematologic</u> or <u>urinalysis</u> parameters. <u>Clinical chemistry</u> - AP and GPT were elevated in the HDT males. Albumin was decreased in HDT males and females. Cholesterol appeared elevated at the mid and high levels in males and females.

	Treat	ment leve	1 (ppm)	
	0	100	500	1500
AP (males)	1.0	1.3	1.3	3.5
GPT (males)	14	15	15	78
Alb (males)	3.01	3.12	3.00	2.66
" (females)	3.45	3.35	3.25	2.88
Chol. (males)	136	140	175	189
<pre>" (females)</pre>	166	147	195	208
Testes wt. (gm)	.0018	.0018	.0017	.0.015

There were no treatment related changes in the other clinical chemistry tests. Organ weights - The testes appeared lighter in the high dose dogs (see above table). No other changes were observed. Gross pathology and microscopic pathology - 1 HDT male (#948) and 1 HDT female (#1019) had evidence of hepatic cirrhosis with hepatic cell necrosis, tubular collapse and increased fibrous connective tissue around the triads. One of the HDT males also had diffuse testicular degeneration.

DISCUSSION: Toxic signs included weight loss in the HDT males, decreased food consumption only at 1500/2500 ppm, alteration in liver function tests in the: HDT males - AP (incr.), GPT (incr.); HDT males and females - Alb. (decr.); MDT, HDT males and females - Chol. (incr.). The elevated AP and GPT were altered in only 1 (#948) of the four male HDT animals which also had advanced hepatic cirrhosis. These changes as well as the increased Chol. in the mid and high dose dogs (male and female) indicate, as the registrant suggests, liver injury probably due to treatment. Testes weights were decreased in 3 out of the 4 HDT males with one of these dogs having mild diffuse testicular degeneration, also suggestive of a treatment related effect. The registrant considered this degeneration reversible but provided no evidence for this conclusion. HDT Males appeared to be more sensitive to treatment with MBC however, this may be a result of receiving about 14 % more MBC per kg body weight than the HDT females.

CONCLUSION: NOEL 500 ppm (.05 %) (14 mg/kg)

LEL 1500 ppm (.15 %) (41 mg/kg), toxicity

consisted of increased alkaline phosphatase, cholesterol and

GPT. The target organs demonstrated by histopathological

changes at the high dose in 1 out of 4 males appeared to be

liver and testes. This is consistent with lesions observed

with Benomyl (the parent compound).

CORE-CLASSIFICATION: minimum

Reviewed by M.P.Copley, D.V.M. Tox. BR. 9/19/85 STUDY TYPH: Acute Neurotoxicity-Hens

TOX. CHEM. NO.: 79C

HASKELL LAB. REPORT NUMBER: HLO 28-79

ACCESSION NO: 241931

IRDC No.: 125-029 (study 1)

125-028 (study 2)

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: International Research and Development

Corporation, Mattawan, Michigan

AUTHORS: E.I. Goldenthal

DATE REPORT SUBMITTED: 4/13/78 (original), 6/5/78 (addendum)

TEST MATERIAL: 2-benzimidazolecarbamic acid, methyl ester (99.3 % Tech., % a.i. not given) (metabolite of Benomyl)

SYNONYMS: MBC

INE-965

MATERIAL AND METHODS: Fasted White Leghorn hens (1108-2254 gm, 6-14 months old) were given by gavage, single doses of the following test materials in 20 ml corn oil/kg:

- 1	1	# tre		T
Compound	Dose (mg/kg)	Study 1	Study 2	Mortality
O(vehical cont.)	n	10	10	0
TOTP(pos. cont.)	750	10	10	1 0
MRC	500	1	10	1
•	2500		10	n
	5000	10		2(day 3)

TOTP - tri-o-tolyl phosphate

They were individually housed in environmentally controlled rooms and given water and food ad libitum. After treatment they were observed daily for pharmacotoxic signs including neurotoxicity and weighed pretest and weeks 1, 2, 3 and 4. All hens were necropsied an examined grossly. Microscopic examination was performed on selected nerve tissue from spinal cord (3 levels) and the sciatic nerve.

RESULTS AND DISCUSSION: Two of the 10 high dose MBC treated (5000 mg/kg) hens died on day 3. The deaths were considered to be the consequence of acute toxicity of MBC. The 1 LDT death was due to accidental injury. Body weight in the TOTP and 5000 mg/kg groups decreased 8-11% from pretest values while there was no significant change for the other groups.

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^{*}Study 1 was initiated 3 weeks prior to study 2.

Signs of toxicity are as follows:

	sl. staxia	Study 1 1/10 (days 15-16)	study 2
TOTP	salivation	5/10 (days 2-3)	4/10 (days 2-3)
	leg weakness, ataxia and/or goose step.	10/10 (days 7-28)	9/10 (days 7-28)
500 MBC	salivation		1/10 (day 2)
2500 MBC	salivation		1/10 (day 2)
5000 MBC	salivation, sl ataxia	.*2/10 (day 2) 8/10 (1-8 days)	
* both of	these died		

Although MBC treated hens showed some neurotoxicity at 5000 mg/kg, only the TOTP treated hens showed symptoms of delayed neurotoxicity. The neurotoxic behavioral symptoms displayed early in the 5000 mg/kg MBC treated hens were attributed to acute toxic effects of the chemical. Hens treated with mid and low doses of MBC (2500 mg/kg and 500 mg/kg, respectively) appeared normal except for some salivation. No treatment related gross pathological effects were seen at sacrifice in the MBC treated hens. There were perivascular lymphoid infiltrates (cervical, thoracic and lumbar cord segments) present in all groups. Axonal degeneration and demyelination were not present in either the vehicle controls or MBC treated hens. Microscopic examination of spinal cord and sciatic nerves showed a spectrum of (expected) positive findings characteristic of TOTP treatment in the TOTP treated hens.

CONCLUSION: MBC does not appear to have delayed neurotoxic potential

NOEL for other neurotoxic signs: 2500 mg/kg

CORE-CLASSIFICATION: guideline

Reviewed by M.P.Copley, D.V.M. Tox. BR. 9/19/85

MP1988333

STUDY TYPE: Two-year Feeding study-Rat

TOX. CHEM. NO.: 79C

HASKELL LAB. REPORT NO: 195-72 MR NO.: 1149 FICHE/MASTER: 00088333 ACCESSION NO.: 232870-232871

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial Medicine, Wilmington, Del.

AUTHORS: H.Sherman, S.R.Fritz, L.S.Wasileski

DATE REPORT SUBMITTED: 1972

TEST MATERIAL: 2-benzimidazolecarbamic acid, methyl ester (50 or 70 % a.i., 53 or 72.2 % Tech.) (metabolite of Benomyl)

SYNONYMS: MRC INE-965

MATERIAL AND METHODS: Male and female albino Charles River-CD strain rats were housed in pairs (by sex) and given food and water ad libitum. After a 12 day observation period (at 33 days of age) healthy rats were divided into groups based on equal average weight. The test compound was then added to the diet by the following scheme for either 1 or 2 years (see necropsy method):

Group	no. male	no. female	PPM	(%a.i.)
group 1	36	36	n	(0)
group la	36	36	n	(0)
group II	36	36	100	(.01%)
group II	36	36	500	(.05%)
group IV	36	36	*2500	(.25%) - 10,000 (1.0 %)
group W	20	20		(.50%)

* level raised to .75% at 18 weeks and again to 1.0% two weeks later ** started treatment 3 weeks later (33 weeks of age) without preliminary hematology

Observations: Animals were observed and examined regularly (interval not specified) for behavioral and toxicological abnormalities.

Food Consumption and Weight: Animals were weighed once/week for 12 months than twice/month for the remainder of the study. Food consumption was monitored for the same intervals by sex and group (except group V).

Laboratory Studies: Hematology - Ten randomly selected rats/sex from groups I, Ia, and IV were tested at pretest (6/sex/group), 1, 3, 6, 9, 12, 18 and 24 months for hematocrit (HCT), hemoglobin (Hg), RRC count, WRC count and WRC differential count. Group V was tested at 18 and 24 months. <u>Urinalysis</u> (UA) - Urine was collected over a 24 hour period from the animals used for hematology (no pretest UA) and examined with respect to the following: protein, sugar, blood, pH, volume, solute concentration (mosmoles/1), color, appearance and microscopic abnormalities. <u>Riochemistry</u>-Ten randomly picked male and female rats from groups I, Ia and IV were tested

t used for the first 8 weeks of the study to used for the remainder of the feeding study

after 1, 3, 6, 9, 12, 18 and 24 months for plasma alkaline phosphatase (AP) and serum glutamic-pyruvic transaminase (GPT). Group V was also tested at 12, 18 and 24 months.

Necropsy: There was an interm 1 year sacrifice with gross and microscopic pathologic examination reducing each sex/group to 30 animals. After 2 years, the surviving rats were also sacrificed. Animals that died or were sacrificed at other than the scheduled times were necropsied and tissues saved for histology when cossible. Tissues were fixed in Bouin's solution, stained with hematoxylineosin and examined microscopically. All listed tissues from contradand group IV (12 and 24 months) were examined, while only the liver from groups II and III and liver, kidney and testes from group V were evaluated at 24 months.

tbrain	tliver	thoracic aorta
theart	tpituitary	thymus
tkidney	epididymis	bone marrow
tadrenal	lymph node	lumber spinal
ovaries	peripheral nerve	trachea
tstomach	Fallopian tube	tlung
eye	tspleen	pancreas
skeletal muscle	thyroid, parathyroid	duodenum
urinary bladder	prostate	cecum
salivary gland	ftestes	colon
exorbital lacrimal gland	uterus	all-masses an '
		abnormal tin:

torgan weights, all groups, at 12 and 24 month sacrifice

RESULTS: Clinical Signs: There were no treatment related signs of toxicity noted in the study however individual animal data was not present to support conclusion.

Mortality: Mortality rate and mean age of death were not treatment related.

Body Weight Gain: There was a decrease in weight gain evident fro-15 months until the end of the study in group V females and group IV males and females.

		of controls
Group	15 months	24 months
V (0.5%) females	86	76
IV (1.0%) females	93	87
IV (1.0) males	94	84

Food Consumption and Efficiency: There were no treatment related differences between control and treatment groups for food consumpt and efficiency.

Dose: Group II and III males and females received approximately the same dose (mg/kg) of MBC. Group IV females however received more MBC (mg/kg) than the males until day 644 at which time they received approximately the same level.

Laboratory Studies: Hematology -

			twenty-fou	r month v	alues	
Group	m	ales			females	
	RBC	HGB	HCT	RBC	HGB	нст
1 (0%)	3.68	ໍ້13.8ີ້	40	6.31	15.0	42
11 (.05%)	3.40	13.2	40	6.33	14.1	39
(0.5%)	3.14	13.2	38	5.23*	12.0*	36*
v (1.0%)	2.92	12.1	36	5.17*	12.0*	36*
significar			ontrols - p	< 0.05		

At 18 months the HCT and HGB in group IV females were lower than controls Statistical significance was not mentioned. However, by 24 months the HCT, HGB and RBC in groups IV and V were significantly (p <.05) lower than controls. Males also had a decreased (not significant) RBC, HGB and HCT. <u>Urinalysis</u> — there were no treatment related abnormalities. <u>Biochemistry</u> — The registrant reported increased AP activity for both males and females at 6, 9 and 12 months in the 1.0% treatment group (12 month females p <.05). At 9 months the SGPT was elevated significantly for both males and females in the 1.0% group and at 12 months for the females.

SURVARY OF BIOCHIMICAL MEASUREMENTS MADY IN RATS FED INE-965 FOR TWO YEARS

			MALE					
	INE-965	NE-965 MONTHS ON TEST						
·	in Diet	1)	6	9	12	_18	74
	0	7.8	3.7),8	1,1	4,5	6.5	9.2
Atkaline Phosphatase	G	7.2	3.7	3.1	3.1	4.5	8.8	•
Bessey Unital)	0.05		•	•	•	4.0	9.6	. 9.4
	1.0	6.9	. 3.9	4.7	4.1	4.8	8.3	8.8
	0.50	•	•	•	•	5.0		7,7
	0	22	23	39	29	31•	26	27
Transaminase	0	26	24	31	39	290	42	•
Unite	0.05	•	•	•	•	37	37	19
	1.0	29	. 26	31	54	31	57	27
	0.30	•	•		•	31	42	13

			THALE					
	THE-965			HOR	ITHS ON 1	EST		
	in Diet	1	3		,	12	18	24
	0	5.8	4.4	3.4	3.5	4.0	6,7	9.2
Alkaline Phosphatase	0	5.7	3.4	3.3	3.2 .	3.7	5.6	•
Bessey Unital)	0.05	•	•	•	•	4.5	5.5	7.4
	1.0	6.8	3.7	4.0	4.0	6.1	7.1	10.4
•	0.50	•	•	•	•	4.7	7.0	7.3
<u> </u>	0	24	28	26	39	18*	47	40
Transeminase .	0	27	23	23	43	33•	60	•
Unita	0.05	-	•	•	•	39	57	33 - '
	1.0	24	30	29	81	49	62	45
	9.50	:	•	•	•	61	83	34

¹⁾ One through 12 months, units/ml blood; 18 and 24 months, units/ml plasms.

^{*} Five animals/group examined.

Pathology: Organ weights - There was no apparant treatment related change in organ weights at either sacrifice time.

Gross - Observations at necropsy were not reported in this study. Microscopic - There was a slight increase in incidence and severity of cholangiohepatitis and pericholangitis in the liver noted in group IV and V males and females. Prostatitialso appeared slightly increased in group IV males (21% incidence as compared to 11% in the controls). Intermediate group rats were not examined histologically for this lesion. There were no other treatment related lesions.

DISCUSSION: There appeared to be no treatment related change in clinical signs and mortality, however 2500 (10,000) ppm females and 5000 ppm males and females gained less weight than control groups. There was no related decrease in food consumption or feed efficiency at these levels. MBC appeared to affect the HGB, HCT and RBC in females at 2500 (10,000) and 5000 ppm and to a lesser extent, HGB and HCT in the high dose males. Although the registrant reported significant increases in AP and GPT in the 5000 ppm group these may not be biologically relevant since: 1) many of the values do not appear out of the normal range for these tests, 2) the elevated means appear only sporadically throughout the 2 year study period. No other biochemical tests were performed, limiting the usefulness of this study. Although AP and GPT were the only biochemical parameters tested there is no reason to expect other changes. Observations taken at necropsy were not noted in the report although they were listed as part of the procedure. Increased incidence and severity of "pericholand and cholangiohepatitis" although not serious, is a toxic effect of the compound. The significance of increased prostati in this study in not known. There may have been a slight increase over controls of pigment deposits in the spleen and bone marrow often observed with decreased hematologic parameter (due to hemolysis) but this was not consistant.

CONCLUSIONS:

NOEL = 500 ppm

LEL = 5000 ppm based on: 1) decreased weight gain in females, 2) decreased HCT, HGB and RCB in females, 3) increase cholangiohepatitis and pericholangitis in males and females.

CORE-CLASSIFICATION:

Although lack of complete clinical chemistry data exists, sufficient histopathology and organ weight measurements permit core-classification of minimum.

Review by M.P.Copley, D.V.M. Tox. Br. 9/19/85 STUDY TYPE: Two-year Feeding study-Dog

TOX. CHEM. NO.: 790

HASKELL LAB. REPORT NO: 195-72

MR NO.: 1149

FICHE/MASTER: 000883 ACCESSION NO.: 23287

2328"

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial

Kedicine, Wilmington, Del.

AUTHORS: H.Sherman, S.B.Fritz, L.S.Wasilecki

DATE REPORT SUBMITTED: 1972

TEST MATERIAL: 2-benzimidazolecarbamic acid, methyl enter (50 or

70 % a.i., >3 or 72.2 % Tech.) (metabolite of Benomyl)

SYNONYMS: MBC

INE-965 carbendazim

Review by Bruce Jaeger in the 1983 WHO report:

"Groups of beagle dogs (four males and 4 females/group) were administered carbendazim (53% active ingredient) in the diet at dosage levels of 0, 100, 500 and 2500* ppm for 2 years. Dogs were one to two years of age at the start of the test. Some dogs in the high dose group received only 1500 ppm. Food consumption and body weight data were obtained weekly, and animals were examined daily for clinical signs of toxicity. Hematological, biochemical and urinalysis examinations were performed periodically* throughout the study. Interim sacrifice after one year was performed on one male and one female from control and 500 ppm groups, as well as one female from the high dose group. One male from the high dose group was sacrificed in extremis after 42 weeks on test diet. Organ weights, gross necropsy and histopathological evaluations* were performed at the conclusion of the study. Only the livers and testes were examined histologically in the 100 and 500 ppm dose groups.

There was no mortality reported for the control or 100 and 500 ppm dose groups. However, three males in the high dose group were sacrificed after 22 and 42 weeks because of poor nutrition. No females in the high dose group died. Body weight and food consumpt were all adversely effected in the high dose group animals, but not at lower levels. The average daily intake for the 500 ppm dose group was 15.0-20 mg/kg (initially, M & F), 14-18 mg/kg (1 year) and 10-16 mg/kg (2 years). Dogs in the highest dose group developed anorexia, distended abdomens and overall poor nutritional condition. Hematological evaluations and urinalyses were not apparently affect by treatment. The dogs in 500 ppm and 1500/2500 ppm dose groups ha increased cholesterol, BUN, total protein, GPT and APase levels while similarly presenting evidence of a decreased A/G ratio through

^{*} see addendum for clarification and additional information.

the study. This biochemical evidence of liver effect was supported by liver pathology, with incidences of hapatic cirrhosis, swollen vacuolated hepatic cells and mild chronic hepatitis in dogs fed 500 ppm or more of carbendazim. There were no noticeable effects on organ weights and organ-to-body weight ratios. Diffuse testicular atrophy (which was marked) and aspermatogenesis were observed in 2/4 males at 100 ppm but were not present in other dose groups or control males. Based on the lack of supporting data in the other dose group males, these findings are not considered compound related.

The NOEL in this study appears to be 100 ppm, based on the liver effects noted at 500 ppm and greater."

Addendum:

MATERIAL AND METHODS: Food was offered ad libitum between 3:00 PM and 7:00 AM. The compound was introduced gradually into the diet of group IV (2500 ppm); 500 ppm for 2 days, 1000 ppm for 3 days, 1500 ppm for 2 days then 2500 ppm. Due to weight loss and decreased appetite the compound level for several dogs was reduced to 1500 ppm. Hematological tests, biochemical tests and urinalysis were performed 3 times pretest, and 1, 2, 3, 6, 9, 12, 15, 18, 21 and 24 months after test initiation. Hematology - Red blood cell count, hemoglobin, hematocrit, total and differential leukocyte counts. Biochemistry - Glucose, urea-nitrogen, cholesterol, alkaline phosphatase (APase), glutamic-pyruvic transaminase activity (GPT), total protein (TP), and albumin/globulin ratio (A/G), albumin concentration. <u>Urinalysis</u> - color, appearance, pH, volume, osmolality, protein, sugar, urobilinogen, acetone, bilirubin, occult blood and microscopic sediment examination. Tissues from the control and 2500 ppm groups were fixed in Bouin's and stained with hematoxylin and eosin for histologic examination included:

tbrain	tadrenal	ileum	mammary gland
theart	tprostate	jejunum	esophagus
tlung	tpituitary	cecum	gall bladder
tliver	rancreas	colon	spinal cord
tspleen	urinary bladder	rectum	trachea
tkidney	epididymis	skeletal m.	salivary gland
ttestis	Fallopian tubes	peripheral n.	tonsil
tthymus	uterus	bone marrow	lymph node
tstomach	ovary	еуe	skin
tthyroid	duodenum	thoracic aorta	

torgan weights were taken

RESULTS:		MALE	S (PPM)	l	FEMAI	LES (PI	PM)
	0	100	500	1500/2500	0	100	500	1500/2500
Chol.(2 mon.)	132	127	174	175	164	157	167	239
(2 yr)	152	150	200	250	211	162	411	171
APase(2 mon.)	1.5	1.2	2.7	13.	2.5	2.9	2.5	7.9
(2 yr)	2.4	2.3	3.3	4.3	3.0	3.8	4.6	6.1
GPT (2 mon.)	18	24	16	138	11	15	22	134
(2 yr)	13	12	17	18	13	10	15	18
Alb/G(2 mon.)	. 90	1.01	.86	.70		no ti	eatmen	t related
(2 yr)	•90	98	1.05	.69		chang	д е	

Increases in (male and female) chol. and APase started as 004679 early as 1 month and remained elevated throughout most of the study in the 500 and 2500 ppm groups. GPT (male and female) increased by 1 month but returned to normal levels within the first year in the 2500 ppm group. Alb/G ratios (males) decreased within 1 month and remained low throughout the study in the high dose group. Regression analysis of chol., APase, GPT, Alb/G and Alb indicated a relation between the level of compound in the food and the change in blood levels. An F test also indicated differences between the treatment groups for these parameters (2 standard deviations were considered significant).

DISCUSSION: The report discussed problems with nutrition at 2500 ppm and 1500 ppm but did not state: 1) which dogs received less than 2500 ppm; 2) how long and how often they were switched to control diets for recovery. This information would be necessary to adequately assess toxicity at the high dose. This deficiency will not change the results of the study however, since major signs of toxicity were also observed at the 500 ppm level. The registrant discussed regression analysis of biochemical values but did not mention which type of analysis was used. The previous reviewer reported increases in BUN and TP, however these changes do not appear biologically relevant because they were within the range of pretest levels or had no dose response. As reported by the previous reviewer and the registrant the histologic and biochemical changes indicated liver damage at 500 ppm and above. There were no other treatment related changes evident in this study.

CONCLUSION:

NOEL = 100 prm

LEL = 500 rim based on biochemical and histological alterations indicating liver dammage.

CORE-CLASSIFICATION: minimum

Original review evaluated and addendum added by M.P.Copley, D.V.M.

Tox. Br.
9/19/85

904675

STUDY TYPE: Reproduction - rat

TOX. CHEM. NO.: 790

HASKELL LAB. REPORT NO: 195-72 MR NO .: 1149

FICHE/MASTER: 00088333 ACCESSION NO.:

232870-A 232871

SPONSOR: E. I. du Pont de Nemours and Company

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial

Medicine, Wilmington, Del.

AUTHORS: H.Sherman, S.B.Fritz, L.S.Wasileski

DATE REPORT SUBMITTED: 1972

TEST MATERIAL: 2-benzimidazolecarbamic acid, methyl ester (50 or

70 % a.i., 53 or 72.2 % Tech.) (metabolite of Benomyl)

SYNONYMS: MBC

INE-965

carbendazim

MATERIALS AND METHODS: ChR-CD rats from the 2 year chronic feeding ... study (HLR 195-72) were used for this study. At 21 days of age they were treated with:

Group	PPM (%a.i.) of MBC	
group 1	0 (0)	
group II	100 (.01%)	
group III	500 (.05%)	
group IV	*2500 (.25%) - 10,000 (1.	0 %)
group V	5000 (.50%)	

*lavel raised to .75% at 18 weeks and again to 1.0% two weeks later

The diets were prepared weekly and refrigerated until used. Mating procedure: Sixteen females and 16 males per group were used for the Fo generation. Each female was mated to each of 3 males for 5 days. Three weeks after mating they were observed twice daily for birth of the F_{1A} litters. All litters were reduced to 10 pups 4 days after birth. The F_{1A} litters were examined at weaning (21 days) and sacrificed. One week later, the F_0 rats were remated to produce the F_{1B} litters. The F_{1B} rats were fed the above mentioned diets after weaning. At 110 days of age 16 males and 16 females were mated using the procedure described previously producing the F_{2A} and F_{2B} litters. F_{3A} and F_{3B} litters were produced using the same procedure with the F2B generation. Parameters examined: Fertility index*, gestation index**, viability index***, lactation index****, weanling pup weight, live/dead ratio.

^{* %} of matings resulting in pregnancy

^{**} % of pregnancies resulting in birth of live litters

^{*** %} of pups that survived 4 days

^{**** #} pups surviving 4 days # pups surviving 21 days (weaning)

Histopathologic examination of kidney, liver, trachea, heart, lung, brain, testis, bone marrow, spleen, thymus, gastrointestinal tract, adrenal, thyroid, pancreas, sciatic nerve and muscle was performed on 2 males and 2 females from each of 5 litters (20 pups total) from the control, 500 and 10.000 ppm groups in the F_{3B} generation at weaning.

RESULTS: All parameters were the same as the controls except average weight of weahlings.

MBC		A v	e. wt	of wea	nlings	(g)		
(ppm)	group	FIA	FlB	F ₂ A	F ₂ B	F3A	F3B	-
0	I	57	60	53	57	53	58	
100	II	60	62	53	62	51	63	
500	III	60	61	55	60	53	62	
2,500*	ΙV	56						
5,000	V	46	52	38	49	39	46	
10,000*	ΙV		41	36	41	39	43	
*see no	te about	diet ch	ange	n grou	pĨVδ	n pre	vious	Þŧ

No histologic or necropsy changes were observed in the study.

DISCUSSION: It cannot be determined from the description when the females were checked for pregnancy, if at all, and if they were all mated to 3 males or only those who were not already pregnant. There were no litter (or fetal) weights taken at birth, only at weaning. It therefore cannot be determined if the pup weight decrease at levels of 5000 ppm and greater is due to toxicity during the prenatal or lactation period. The number of dams in the test was only 16 (20 for group V), resulting in only 10-16 litters per group rather than the 20 litters recommended in the guidelines. This study, however, did have 6 matings (3 generations) per group which excedes the requirements. Due to the known testicular effect of MBC and Benomyl, special attention should have been given this organ such as organ weights. Nevertheless, histopathological examination of weanlings in the F3B generation (500 and 10,000 ppm groups) showed no testicular lesions.

CONCLUSION: NOEL = 500 ppm

LEL = 5000 ppm based on toxic signs of decreased pup weight noted at weaning.

CORE-CLASSIFICATION: minimum

Review by M.P.Copley, D.V.M. Tox. Br. 9/21/85